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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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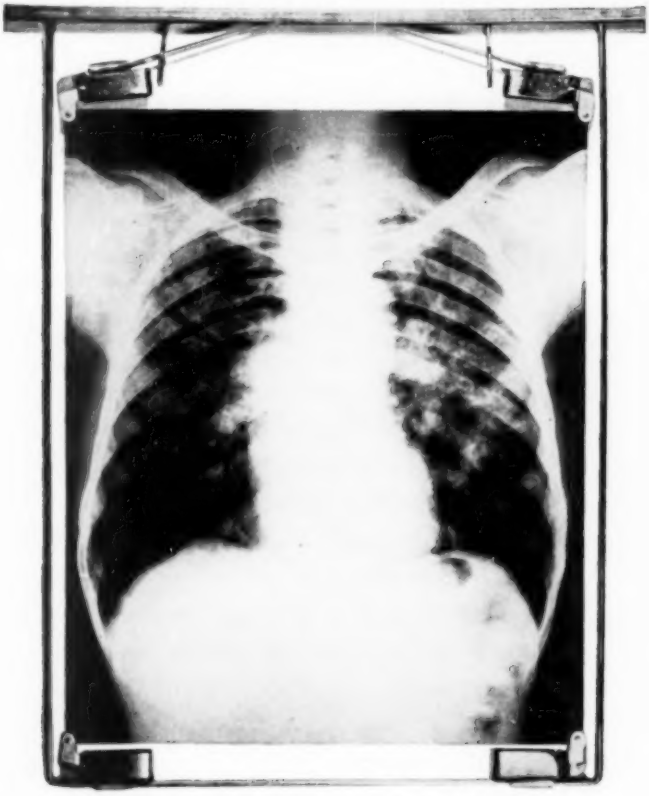

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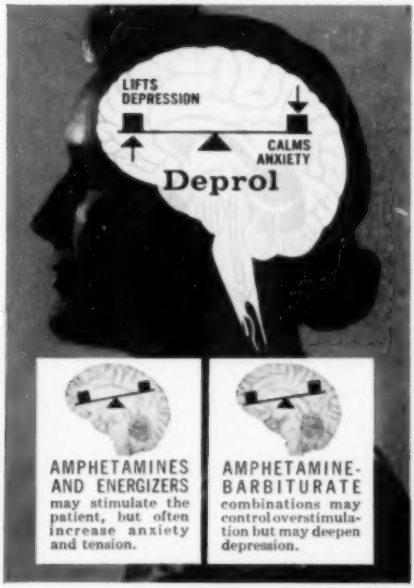
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Registration will be in the Statler Hilton and will begin at 9:00 AM, SUNDAY, MAY 15.

Exhibits will be in the Statler Hilton and will open for viewing at 8:30 AM, MONDAY, MAY 16.

Special interest sessions will begin SUNDAY, MAY 15. *Medical, public health, and nursing sessions* will begin MONDAY, MAY 16.

General sessions will include an opening keynote program Monday at 9:00 AM, followed immediately by the awarding of the Trudeau and Will Ross medals; a session on Monday afternoon at which results of the Arden House Conference on Tuberculosis will be presented (the opening portion will be of special interest to doctors); a session Tuesday morning, and the NTA Business Luncheon on Thursday.

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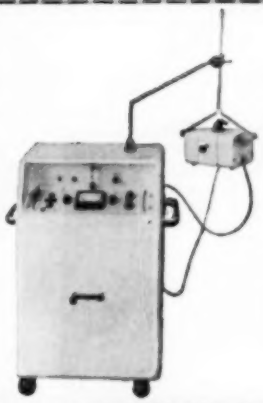

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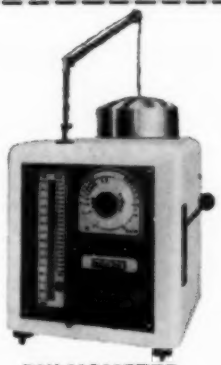

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THE AMERICAN REVIEW
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VOLUME 81 *January 1960* NUMBER 1

STUDIES OF HUMAN PATTERNS OF ISONIAZID METABOLISM USING AN INTRAVENOUS FALL-OFF TECHNIQUE WITH A CHEMICAL METHOD[1]

JOHN W. JENNE

(Received for publication July 21, 1959)

Introduction

It is well established that the levels of bacteriologically active isoniazid in serum, as a function of time following an oral dose, vary significantly between individuals and racial groups (1, 2). Approximately one-third of persons of European descent are found to have serum concentrations of free isoniazid below 0.4 γ per ml., and three-fifths above 0.8 γ per ml., six hours after an oral dose of 4 mg. of the drug per kg. (3). Analysis of free isoniazid in the urine reveals a positive correlation with the serum concentrations (1, 4). On the basis of work with pigeon liver *in vitro* (5), and the study of urinary products in humans (6), it has been assumed, but not proved, that the differences in serum concentrations of isoniazid in humans are largely the result of variations in rate of acetylation by the liver. The patients with low six-hour concentrations have been termed "rapid inactivators." The clinical significance of such grouping with respect to response to chemotherapy is under study in many centers. Preliminary reports have suggested that treatment failures or delayed responses to chemotherapy, and persistent excretion of isoniazid-susceptible organisms, are more frequent in the "rapid inactivation" group (3, 7).

The most commonly employed method for measuring free serum isoniazid utilizes a bioassay measurement based on serial dilutions, and indicated by growth inhibition or loss of acid-fastness of a susceptible Mycobacterium (8, 9). This approach has been of undoubted value, and is particularly suited to measurement of the low values at six hours. However, there are certain recognized weaknesses of such a bioassay technique. Among these are the delay of approximately two weeks for receipt of the results and the necessary technical provisions, often prohibitive for the small hospital. In addition, its use in investigation is severely limited because serum concentrations are reported in a range, usually a twofold dilution.

Several chemical methods have been developed in an attempt to overcome these disadvantages (10–13), but these methods are at a disadvantage at low six-hour levels.

The present paper proposes a new technique which employs a rapid intravenous injection of isoniazid to obtain high, easily measured, and immediately differentiable fall-off patterns of the metabolism of the drug. It utilizes, with slight variations in technique, the recently published method of Poole and Meyer (12) for the determination of free isoniazid in serum. This technique appears to offer certain advantages over previous methods. First, it provides a pattern of metabolism prior to commencement of therapy; second, it is simple, requiring a minimum of equipment; and, third, it is believed accurate enough to be used in the study of possible inhibitors of inactivation and the influence of various clinical circumstances.

Materials and Methods

The patients were tested in most cases prior to the commencement of treatment for active tuberculosis. None were acutely ill. If patients were on therapy, drugs were discontinued 36 to 72 hours prior to the test. Only essential medications were allowed the evening before. Breakfast con-

[1] From the Pulmonary Disease Service, Veterans Administration Hospital, and the Department of Medicine, University of Minnesota, Minneapolis, Minnesota.

sisted of toast and coffee. A 15-ml. quantity of blood was drawn for the blank determination and, using the same needle, 5 mg. of isoniazid[2] per kg. were given intravenously during a three-minute period. A 15-ml. sample of blood was drawn at 30, 90, and 150 minutes after the mid-point of the injection. Each serum was set up in duplicate. Following alcohol precipitation, the tubes were shaken well by hand, centrifuged, and the contents filtered in order to clear a frequent fine turbidity. Suitable standards demonstrated compliance with Beer's law, confirming the original report. In most cases, a standard of 10 γ per ml. was prepared in pooled serum along with its own blank. This step was unnecessary for the half-life determinations, however, since measurement of relative optical density is sufficient when measuring the rate of decay. The resulting chromogen was measured in a Bausch and Lomb Spectronic 20 colorimeter at 500 μ against the patient's pre-medication blank. The optical density was transferred directly to the log scale of two-cycle log paper, averaging the duplicate determinations at each interval. When a difference between duplicate blanks of more than 0.01 density units was encountered, the samples were read against each blank, the fall-offs plotted, and the half-lives averaged. A straight line was usually plotted through the three points with no difficulty, indicating a close approximation to a first-order decay curve. It was unnecessary to use statistical methods for the best fit, since any such improvement in accuracy was of no practical consequence. The values in the rapid fall-off group were found to show more apparent deviation from a straight line in the low concentrations. However, the line was drawn so that deviations from the line at each interval were approximately equal in optical density units.

As a confirmation, blood was drawn from 13 patients at 240 minutes for bioassay. A few patients were also given an oral isoniazid-tolerance test of 4 mg. per kg. but, in most cases, the consequent delay in therapy in the acute stage of their disease was thought inadvisable.

Six patients had repeat fall-off determinations with the same dose after one or two months of treatment with isoniazid, with or without another drug. Six other patients had repeat tests with twice the usual dose, using 10 mg. per kg. of isoniazid. A number of patients with abnormal liver function, but without edema, were tested. Several of these had serious manifestations of cirrhosis, including one who was tested before and after a portacaval shunt procedure. The others had abnormal bromsulphalein retentions (over 5 per cent in 45 minutes) and a history of excessive alcohol intake. Other causes of abnormal liver function were excluded insofar as possible. One patient was tested twice during the development of congestive failure. Bromsulphalein retention, serum bilirubin, blood urea nitrogen, and phenolsulfonphthalein excretion tests were done on most patients to serve as approximate indicators of normal renal and hepatic function. Three patients with suspected "rapid inactivation" because of suboptimal clinical response and one known "rapid inactivator" were also tested.

In order to determine the standard deviation of the chemical assay at high and low concentrations of isoniazid, standards of the drug were prepared so as to give calculated concentrations in serum of 10 γ per ml. and 2.0 γ per ml. Ten specimens of each were measured. The 10-γ per ml. determinations gave a standard deviation of 0.29γ per ml. The 2.0 γ per ml. specimens, standardized against this, gave a mean value of 2.15 γ per ml. $\pm$ 0.16 γ per ml.

Results

The combined results of the first determinations in 43 patients are summarized in table 1. The half-lives in minutes are tabulated and the patients are somewhat arbitrarily divided into two groups, with half-lives ranging from 45 to 110 minutes and 110 to 240 minutes. The percentage of bromsulphalein retention at 45 minutes is listed for each patient. Bioassay determinations are listed where obtained, and reported as the lower level of a twofold dilution. There is seen to be a clear-cut division of these values between the two groups with the exception of patient *J..A.*, whose reliability in withholding his noon oral medications was questioned. Isoniazid concentrations extrapolated to "zero-time" are listed in those cases in which standards were done.

There was no correlation between zero-time concentration and separation into the above group. The calculated mean concentrations were 8.0 $\pm$ 1.9 γ per ml. and 8.0 $\pm$ 1.7 γ per ml., respectively. No correlation was found between initial concentrations of isoniazid and half-lives *within each group*, or initial concentration and volume of drug injected.

The variation in zero-time concentrations might be ascribed to deviations from the calculated quantity of the actual quantity injected due to variations in potency of the isoniazid solution in the ampoules or to syringe errors. Four milliliters of drug solution were drawn with a separate syringe from each of ten ampoules and diluted to a calculated concentration in serum of 8.0 γ per ml. These were measured in duplicate. As standardized against the 10.0-γ per ml. standard already described, these determinations gave

[2] Given as Nydrazid Injection, manufactured by E. R. Squibb & Sons, New York, New York. The preparation is stable and contains 10 ml. of isoniazid at a concentration of 100 mg. per ml., and 0.5 per cent chlorbutanol.

TABLE 1

TABULATED DATA OF INITIAL HALF-LIFE DETERMINATIONS IN 43 PATIENTS

5 Mg. of Isoniazid per Kg. Intravenously

Patient	Half-Life	Bromsulphalein Retention	Concentration of Isoniazid at "Zero-Time"	Four-Hour Bioassay
	(*minutes*)	(*per cent*)	(γ *per ml.*)	(γ *per ml.*)
W.H.	455	1.8	7.4	2.4
J.F.	146	3.2	7.9	
W.L.	171	3.5	9.3	1.2
W.B.	180	1.8	7.0	0.6
K.H.	155	2.5		
A.B.	162	5.8		0.6
S.P.	150			
M.C.	141	4.5		
H.M.	155	29.0		0.6
L.E.	225	22.3	12.1	1.2
W.O.	164	2.5	10.9	0.6
R.P.	235	7.5	6.8	0.6
V.C.	240	2.8	6.8	
D.W.	204	1.0	8.2	
J.L.	114	12.0	5.6	
W.N.	238	1.5	7.3	
J.H.	199	6.8	8.2	
K.G.	204	6.0	8.1	
E.M.	120	3.0	6.7	
G.C.	249	1.8	7.2	
G.C.	56	2.8	8.6	
H.B.	63		9.4	
R.H.	71	15.5		
L.S.	68	10.8		Less than 0.15
J.F.	72	3.8		
R.H.	53	2.0		
A.P.	75	3.5		
E.M.	54	3.0		
K.K.	61	2.5		
W.G.	53	17.0		0.15
O.B.	52	9.5		0.15
O.G.	54	7.0	8.0	0.15
J.A.	91	19.3	9.9	1.2
L.P.	81	2.0	9.7	
P.D.	59	1.8	7.9	
H.W.	46	0.5	7.0	
J.J.	45		9.4	
J.L.	94	3.0	5.2	
S.O.	45		5.3	
E.G.	60	17.5	11.6	
B.B.	77	3.7	7.1	
R.V.	78		6.3	
C.B.	107	1.8	6.4	

a mean value of 8.16 ± 0.3 γ per ml. It was concluded that the zero-time variations had their origin in individual patient factors.

The manner of plotting and analyzing the results in a case from each group is shown in figure 1.

Figure 2 shows the distribution of half-lives and categorizes them into unselected patients with normal liver function, patients suspected or known to be rapid inactivators, and patients with abnormal liver function. The bromsulphalein retention is indicated with the last group in parentheses. Most half-lives fell into the relatively narrow limits of 45 to 80 minutes (mean of 59) and 140 to 180 minutes (mean of 159). Eight of the 27 unselected patients with normal liver function had half-lives wide of these limits, however. Three suboptimal-response patients fell in the 45- to 80-minute group. The patient with congestive cardiac failure is not included in figure 2 and will be discussed separately. Six of 12 papatients with abnormal liver function were wide of the above limits, but not in relation to their bromsulphalein retention. It is evident that the 45- to 80-minute group is a distinct group, comprising almost half of all patients tested, and would qualify for the term "rapid inactivation."

Patients with repeat determinations are listed again in table 2, with both values. The patients were either retested with the same or twice the original dose. In either case, the repeat half-lives were close to the initial values in both the rapid and slow groups, with the exception of patients *C.K.* and *W.H.* It was found that doubling the dose approximately doubled the zero-time concentration of isoniazid and consequently the velocity of fall-off at zero-time, V_0,[3] using the y-intercept as the substrate concentration.

C.K., a patient with the recent onset of congestive cardiac failure, had a progressive rise in venous pressure and circulation time over the period of tests. His fall-off velocity at zero-time was actually less on the second test, despite twice the dose of isoniazid.

R.H., a severe cirrhotic recovering from three episodes of esophageal bleeding and pulmonary

[3] $$k_r = \frac{\ln 2}{T_{1/2}} = \frac{0.693}{T_{1/2}}$$

$$V_0 = k_r\,(\text{isoniazid})_0 = \frac{0.693\ (\text{isoniazid})_0}{T_{1/2}}$$

where k_r equals the rate constant of decay in $\frac{\%}{100}$ min.$^{-1}$ and V_0 equals velocity of reaction at zero-time in γml.$^{-1}$ min.$^{-1}$; $(\text{isoniazid})_0$ equals concentration of isoniazid in γml.$^{-1}$ at zero-time; $T_{1/2}$ equals half-life in minutes.

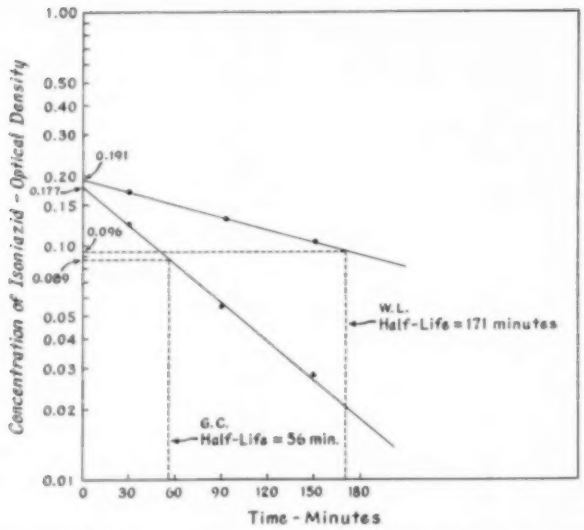

FIG. 1. Method of half-life determinations: 5 mg. of isoniazid per kg. intravenously.

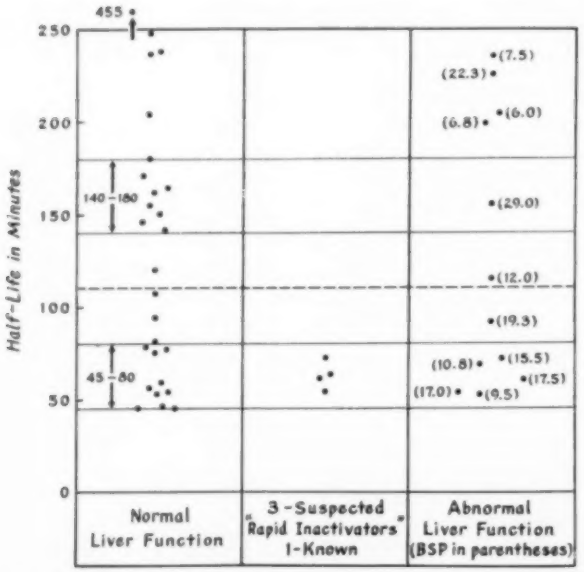

FIG. 2. Distribution of initial half-life determinations: 5 mg. of isoniazid per kg. intravenously.

tuberculosis, was tested with the same dose before and after a portacaval shunt procedure and reduction of portal pressure to normal. Despite the shunting procedure, the half-life remained essentially the same, with successive values of 71 and 60 minutes.

TABLE 2

RESULTS OF REPEAT FALL-OFF DETERMINATIONS (Half-Lives in Minutes)

Same Dose of Drug

Rapid Group			Slow Group		
	5 Mg. per Kg.			5 Mg. per Kg.	
R.H.	53	56	J.F.	146	199
A.P.	75	73	W.L.	171	153
R.H.*	71	60	W.H.	455	640

Single and Double Dose of Drug

Rapid Group			Slow Group		
	5 Mg. per Kg.	10 Mg. per Kg.		5 Mg. per Kg.	10 Mg. per Kg.
H.B.	63	80	K.H.	155	165
C.K.†	49	131	H.M.*	155	173
W.G.*	53	59			
E.M.	54	69			

* These patients had cirrhosis.

† This patient developed congestive failure.

An unusual result was found in patient *W.H.*, and is plotted in figure 3. This patient was a 30-year-old man with tuberculous pleuritis and minimal effusion. He was not an alcoholic, and was otherwise in good health. His first half-life determination was 455 minutes, and three months later the half-life determination was 640 minutes. The values for bromsulphalein retention, serum bilirubin, blood urea nitrogen, serum proteins, and serum cholesterol were normal; serum protein electrophoresis showed changes consistent with subacute infection. The patient manifested no signs of toxicity on 300 mg. of isoniazid daily. It is a reasonable assumption, however, that this patient would develop excessively high and possibly toxic levels of isoniazid on high-dosage therapy. This patient's half-life calculations illustrate the fact that, when dealing with the slower fall-offs, small variations in the slope of the fall-off are reflected in large variations in half-life calculation.

DISCUSSION

The intravenous fall-off technique as here employed and measured is thought to possess the following advantages over the conventional oral bioassay method:

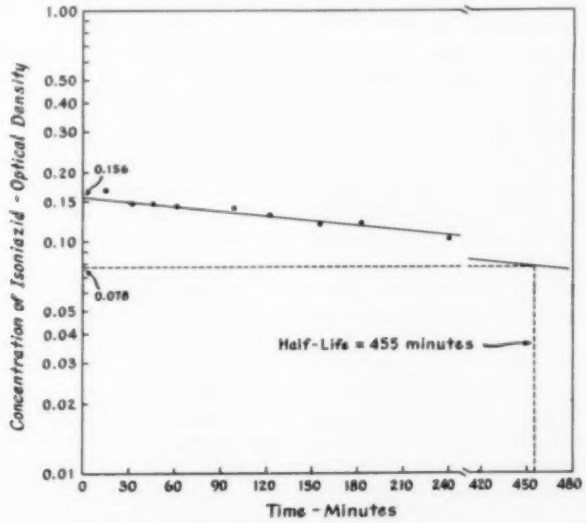

FIG. 3. Isoniazid fall-off in patient *W.H.*: 5 mg. per kg. intravenously.

It eliminates the variable of gastrointestinal absorption in separating the two main groups.

It provides the high concentrations of drug in the blood necessary for the more exact analysis that can be obtained by a chemical method.

It provides immediate information before therapy.

It can be done using the laboratory facilities of the average hospital.

It determines the entire metabolism pattern leading to more accurate appraisal of individual rate variations.

The chief disadvantage of the technique is that it fails to measure actual drug concentrations attained following oral administration. Thus, certain subjects might maintain lower concentrations without displaying increased rates of degradation, and possible variables of gastrointestinal absorption are not taken into account. However, these aspects are believed unlikely to be of major importance. No side effects whatever have been observed in the patients during the tests.

That the results are reproducible in the individual case is a necessity in establishing the validity of the test. The numbers retested are too few to permit calculation of the error of the method, but repeat testing with the same or twice the dose indicated that group assignment is reliable.

Assuming a first-order decay, the area under the decay curve, termed the time-concentration drug exposure, is proportional to the half-life and to the initial drug concentration. Integration of the area under the curve results in the formula

$$\text{Area} = \frac{(\text{isoniazid})_0}{K_r}$$

With the intravenous administration of equal doses, the mean time-concentration drug exposure for the 140- to 180-minute group is approximately two and one-half times that of the 45- to 80-minute group. Unfortunately, this calculation cannot be applied to drug exposure following oral administration.

There remains some uncertainty whether the fall-off of free isoniazid concentrations can be equated with similar variation in antibacterial activity. Furthermore, the antibacterial activity which may be possessed to a slight degree by the various metabolites of isoniazid is ignored by the measurement of free isoniazid. Therefore, samples were drawn during the fall-off at 240 minutes to determine whether bioassay indicated an appropriate qualitative difference between the two groups. The comparison is seen to hold up well.

A comparison of the intravenous chemical method with the oral bioassay method has not yet been adequately studied. However, it seems unlikely that the major differences between the groups result from absorptive differences in the gut.

A fundamental question is whether rate differences are the result of differences in quantity of an enzyme which controls a common rate-dominating reaction. It was hoped that an increased load of isoniazid might reveal some tendency to saturation in the slow group, compatible with such an hypothesis. Patients retested with twice the dose showed, however, an essentially linear velocity response to the increased concentration. This indicates that the predominant process in both groups is far from saturated in this dosage range, and offers no help in resolving the question. Other possible causes of the difference include one or more different conjugation mechanisms and protein-binding differences. The role of the kidney must also be explored.

The results in patients with reduced liver function are somewhat suggestive of a lack of conformity to the distribution of half-lives in normal persons. The data obtained, however, do not establish a significant difference, and it is clear that the 45- to 80-minute group is largely or entirely unaffected by cirrhosis.

Summary

An intravenous fall-off technique for determining individual patterns of isoniazid metabolism is described. It utilizes a previously described, simple chemical method for the determination of free isoniazid. It is believed that the technique offers both clinical and investigative advantages over existing methods. Most of the patients studied to date fall into two groups, one with a mean half-life of 59 minutes (45 to 80), and the other with a mean half-life of 159 minutes (140–180). The rate-dominating process is shown to be approximately first-order, and far from saturated in both groups. A group of patients with abnormal liver function was tested, and the results were suggestive of a slight delay in some of those with slower fall-offs. The delays would seem to be too small to be of any clinical importance.

Circulatory congestive failure is seen to prolong the half-life. Extremely prolonged half-lives of 455 and 640 minutes were recorded in one apparently normal patient. It can be calculated that during the intravenous test the slow-normal group has approximately two and one-half times the time-concentration drug exposure of the rapid group, and that this product is proportional to the half-life and the initial drug concentration.

Sumario

Estudios de los Patrones Humanos del Metabolismo de la Isoniacida Usando una Técnica de la Declinación Intravenosa con un Método Químico

Se describe una técnica de la declinación intravenosa para determinar distintos patrones del metabolismo de la isoniacida. Utiliza además un sencillo método químico, ya descrito, para la determinación de la isoniacida libre. Opínase que la técnica, comparada con los métodos existentes, posee ventajas tanto clínicas como investigativas. La mayoría de los enfermos estudiados hasta ahora corresponden a dos grupos: uno con una hemivida media de 59 minutos (45–80) y el otro con una hemivida media de 159 minutos (140–180). Según se demuestra, el proceso que domina la tasa es aproximadamente del primer orden y dista mucho de la saturación en ambos grupos. Se comprobó un grupo de enfermos que tenían anomalías de la función hepática y los resultados señalaron un leve retardo en algunos de los que tenían declinaciones más lentos. Los retardos parecen ser demasiado pequeños para que posean importancia clínica. Se considera que la insuficiencia circulatoria congestiva prolonga la hemivida. Se registraron hemividas sumamente prolongadas de 455 y 640 minutos en un sejuto aparentemente normal. Cabe calcular que durante la prueba intravenosa el grupo lento-normal muestra aproximadamente una exposición a la droga dos veces y media mayor en tiempo y concentración que el grupo rápido, y que este producto es proporcional a la hemivida y a la concentration inicial de la droga.

Resume

Etude chez l'homme d'un mode de metabolisme de l'isoniazide par une technique intraveineuse basée sur la desintegration chimique du produit

Les auteurs utilisent une methode chimique simple, précedemment décrite qui consiste à evaluer l'isoniazide libre. Cette technique offre, pense-t-on, des avantages cliniques et scientifiques par rapport aux méthodes préexistentes. La plupart des malades etudiés jusqu'à ce jour comporte 2 groupes: un avec un temps de demi-destruction de 59 minutes, (45–80), et l'autre avec un temps moyen de 159 minutes (140–180). Il apparait que le processus dominant est loin d'être un processus de saturation. On a examiné un groupe de malades ayant une fonction hépatique anormale; les résultats semblent indiquer un léger retard chez ceux ayant les desintégrations les plus lentes. Ces retards semblent trop insignifiants pour avoir une importance clinique. La décompensation cardiaque semble prolonger le temps de demi-destruction. Des temps très prolongés, de 455 et 640 minutes ont été rapportés chez des sujets normaux. On peut calculer que pendant le test intraveineux, le groupe ayant un retard normal concentre deux fois et demi plus que le groupe à temps rapide. Le produit est proportionnel au temps de demi-destruction et à la concentration du medicament.

Addendum

Recently the data have been analyzed to determine whether the 150-minute value by itself is adequate to distinguish the two broad groups. The few overlapping values (3 of 39) are from those patients with intermediate values of half-life determination. The dividing concentration is 3γ per ml. in the patients analyzed thus far. This single determination would have value in separating the groups, but would have limited usefulness for clinical studies.

Acknowledgments

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REHABILITATING THE TUBERCULOUS IN ISRAEL[1]

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Introduction

Tuberculosis started becoming a major health problem for Israel in 1949, shortly after the establishment of the state, and reached its peak in 1952. For, by this time about 735,000 immigrants, who had had almost no previous medical or social screening, had come to Israel.

To help the country cope with the general problem of caring for the needy sick, handicapped, and aged new immigrants, the American Joint Distribution Committee, which in the United States receives its funds through the United Jewish Appeal, was asked to assist the Israel government and the Jewish Agency in setting up services to care for them. The organization that evolved in 1949 became known as Malben-JDC (Malben stands for the inital letters of the Hebrew name which means: Institutions for the care of handicapped immigrants), and it has been providing clinical and hospital services for the sick and homes for the aged new immigrants ever since.

The new immigration brought in some people who had been through great difficulties. Many had gone through the concentration camps, and the tortures, hunger, and fear had seriously undermined their health. Others were survivors of the Jewish communities of Poland, Rumania, and other European countries. During the war, many of these people had lived in the forests and still bore the marks of severe physical and emotional suffering. Moreover, since the war, most of them had been cared for in refugee camps in Germany and Italy, and had become used to being supported by relief organizations and to leading unproductive lives.

The largest group of immigrants came from the oriental countries of North Africa, the Yemen, Turkey, Iraq, and Persia. They arrived sick and destitute, having lived all their lives in undescribable misery and poverty in medieval ghettoes. Most were illiterate or had only minimal education, but no skills. In their countries of origin they had lived by peddling, being in small trade, or doing manual work. And even those who considered themselves skilled workers could not qualify by local standards.

These suffering people, with their past misery and broken homes and their then temporary living quarters in tents and canvas huts, represented fertile soil for the spreading of tuberculosis. It was also not surprising that many of the 7,530 immigrants who were found to have active tuberculosis also showed serious emotional disturbances of the kind that often accompany this disease.

It is very difficult to treat such patients, and even more difficult to rehabilitate them. At the beginning the physicians were concerned primarily with treating the lesions of tuberculosis by radical or conservative methods; and it was impossible to create the right atmosphere for rehabilitation within the hospital walls. Therefore, it was decided to open a rehabilitation center for the main purpose of setting up a model rehabilitation plan to foster a better understanding of all the problems connected with the rehabilitation of tuberculous patients. It was hoped that in time the success of the plan would free hospital beds for those in need of active medical treatment. Accordingly, the Rehabilitation Center for Tuberculous Patients was opened on July 1, 1951, at Neve Chaim, about 50 kilometers from Tel Aviv.

Observations

General Data on the Rehabilitation Center

Between the years 1951 and 1957, a total of 299 patients was accepted by the Center, 204 (68 per cent) of whom were men and 85 (32 per cent) women, with 57 per cent less than twenty-five years of age, 35 per cent between twenty-five and thirty-five, and 19 per cent more than thirty-five. As to education, 13 per cent had had none at all, 15 per cent were below elementary school level, 61 per cent had gone through elementary school, and 11 per cent had

[1] From the Rehabilitation Service of the Malben-JDC Hospital for Tuberculosis at Beer Yaacov, Tel-Aviv, Israel.

graduated from high school. Of those with elementary and high school education, 81 per cent were of East European origin (53 per cent of the total number of patients); while 96 per cent without education had come from the Moslem countries.

Medical Data

At the beginning, only patients with stabilized pulmonary lesions were admitted to the Center. From 1954 on, admissions included patients with nonstabilized pulmonary processes assumed to be of low infectiousness or those who had residual cavitation.

Following is a classification by diagnosis:

Pulmonary tuberculosis, 95.97% (287 patients)

extrapulmonary tuberculosis, 4%:
- Tuberculosis of bone, 2.69% (8 patients)
- Tuberculosis of lymph nodes, 1.0% (3 patients)
- Tuberculosis of the genitourinary system, 0.34% (1 patient)

The extent of the disease and the types of lesions are shown in table 1. As may be seen in the table, in 61 per cent of the patients, the tuberculosis could be classified as moderately advanced; while, in 35 per cent, the disease was far advanced. From a clinical point of view, the patients could be divided into those with active disease (27 per cent) and inactive disease (73 per cent). In the last group, the disease was apparently quiescent in 63 per cent while, in 10 per cent, it was considered to be arrested. The patients with arrested disease were in need of vocational and emotional rehabilitation.

To present a complete picture, it is important to note the hospitalization periods prior to admissions to the Center: 20 per cent of the group had been in various hospitals for more than six years; 60 per cent for three years and more; while the remaining patients had been hospitalized for only short periods. Prolonged hospitalization had left its mark from both the medical and emotional-social points of view.

As to the relapses which had occurred in a number of the patients prior to their admission to the Center, it is worth noting that more than three-quarters of the total number of patients had been accepted following past exacerbation of pulmonary tuberculosis, and half of these had experienced two or more relapses. Contrary to the opinion of Kiefer and others that no successful rehabilitation could be accepted by a patient who had suffered more than one relapse, the trainees successfully went through the whole rehabilitation process.

In addition to tuberculosis, many of the rehabilitation trainees were suffering from other diseases at the same time, as may be seen in table 2.

The trainees received treatment as follows:

Temporary collapse	34.5% (99 trainees)
Permanent collapse	15.7% (45 trainees)
Resections	18.9% (54 trainees)
Bed rest and drug treatment	28.9% (83 trainees)
Bed rest alone	2.0% (6 trainees)

While testing the working capacity, the supervision was very strict, since the evaluation had to be made individually in each case. Patients were found whose pulmonary process and lung function, from the age point of view, were similar; yet their working capacities were not. The personality and stamina of the patient were the deciding factors. On admission, an average of 56 per cent of the patients had working capacities of three hours a day, and the capacity of the remainder ranged up to five hours a day.

During the time the patient-trainees were at

TABLE 1

EXTENT AND TYPE OF LESION ON ADMISSION

Type of Lesion	Minimal	Per Cent	Moderately Advanced	Per Cent	Far Advanced	Per Cent	Total	Per Cent
Infiltration without cavitation	—		5	2.83	2	1.94	7	2.43
Fibrosis with cavitation	—		10	5.65	26	25.24	36	12.55
Fibrosis without cavitation	7	2.44	162	91.52	75	72.82	244	85.02
Total	7	2.44	177	61.68	103	35.88	287	100.00

TABLE 2

OTHER DISEASES COMPLICATING TUBERCULOSIS

Pulmonary (not tuberculosis)	
Bronchiectatic lung disease	2
Cystic lung disease	2
Bronchial asthma	4
Sarcoidosis	1
Endocrine and metabolic	
Thyrotoxicosis	3
Cushing syndrome	1
Diabetes mellitus	2
Diabetes insipidus	1
Digestive tract	
Ulcus duodeni	2
Ulcus ventriculi	1
Appendicitis	2
Genitourinary tract	
Nephritic syndrome	1
Nephrolithiasis	2
Myoma uteri	1
Psychiatric	
Schizophrenia	1
Heart and blood vessels	
Mitral stenosis and incompetence	3
Congenital heart disease	2
Tachycardia paroxysm	1
Hypertension	2
Thrombophlebitis	1
Blood-forming organs	
Leukemia myeloid, chronic	1
Gall bladder	
Cholecystitis	3
Rheumatic	
Polyarthritis rheumatica	2
Nervous system	
Hemiplegia	1
Epilepsia Jackson	1
Miscellaneous	
Menier syndrome	2
Amyloidosis	1
Total	46

work, a constant check was maintained on their clinical condition, the extent of their fatigue, and their respiratory rate. Special care was given to the patient's sense of well-being as his workload gradually was increased. It is interesting to note that about 40 per cent of the trainees, upon reaching the capacity of five working hours, could make no further progress for two to three months thereafter. However, the point was reached at which, upon leaving the Center, 86 per cent were able to work seven hours; only in 10 per cent of the cases had the work tolerance remained at three hours, and most of these patients were returned to hospitals.

Vocational Training

Vocational training was started more or less at the same time as the medical rehabilitation. In selecting a trade for the patient, his inclination, the extent of his education, results of psychotechnic tests, and his medical condition were all taken into consideration. Moreover, trades were chosen for which there were openings on the labor market, such as office work, bookkeeping, sewing, carpentry, and metal turning (lathe work).

The average course ran ten and one-half months, and on completion the trainee took the General Federation of Labor (Histadrut) examination. Despite obvious handicaps, such as deteriorated physical condition, unequal education, different study hours which varied with the medical condition of the trainee, et cetera, the graduates of the Center achieved higher grades than did graduates of other schools. This may well have been due to the strong determination of the patients and the individual training they had received.

Economic and Social Problems

Among the trainees, 78 per cent were heads of families and without means other than the social welfare support, which amounted to IL 40, (about $20) a month for a family of eight.

Housing was not much better: 44 per cent lived in temporary reception camps (ma'abarot); 34 per cent, in huts and cellars unfit for tuberculous patients and their large families; while only 22 per cent had suitable housing.

With these difficult living conditions and the lack of employment, many of the trainees had developed to a marked degree the severe emotional disturbances which sometimes accompany tuberculosis. Many of them suffered from depression, dependency, lack of confidence and, particularly, fear of the possible relapse of their disease. In addition, there were often the well-known conflicts that can occur within a family (during the illness, one of the marriage partners becoming attached to someone else), and there were problems of divorce and of children. Special attention was given to each case by the team, and long talks were held with the trainees.

To help the trainee forget his outside worries, a recreational activity program was developed and a recreation committee elected from among the trainees. Various groups were organized, including one for Bible study and another for

technologic studies. These cultural activities provided the trainee with an outlet for his dormant energy, which was slowly reawakening. In addition, there were discussions with the Center's team, outings in the vicinity, and short leaves which brought the trainee closer to the questions of tomorrow and his discharge.

Discharge Problems

After his medical condition had become stabilized and his vocational training was completed, the trainee was ready to leave the Center. At that point the emotional reactions usually became much more pronounced, and there was the danger that, if suitable housing and employment were not provided, he might lose the emotional and physical balance he had achieved.

Due to the efforts of the Center, 68 per cent of the trainees obtained suitable housing, 88 per cent were placed in skilled jobs, 7 per cent were placed in jobs outside their trade, and only 5 per cent could not be placed at all. The distribution by type of employment was as follows:

Office work and bookkeeping
123 trainees: 92% in skilled jobs
5% in other jobs
3% could not be placed

Sewing
51 trainees: 81½% in skilled jobs
11½% in other jobs
7% could not be placed

Turners
48 trainees: 91% in their trade
6½% in other jobs
2½% could not be placed

Carpentry
35 trainees: 80% in their trade
8½% in other jobs
11½% could not be placed

Along with the improvement in the social conditions and the emotional equilibrium of the trainees, the status of their health also gradually improved. The rehabilitative status of the trainees upon discharge from the Center is shown in table 3, as compared with what it was at the time of their admission. Trainees who left the Center before completing their rehabilitation process were not included in this table. Of the 224 patients who were rehabilitated, 236 suffered from pulmonary tuberculosis (and this table deals with this group), 6 from bone tuberculosis, and 2 from tuberculosis of the lymph nodes.

The table shows that 3 patients with noncavernous infiltration and 3 with cavernous fibrosis successfully reached the stage of noncavernous fibrosis. Only one patient continues to suffer from cavernous fibrosis.

It is interesting to note that 5 of the 18 patients suffering from the complicated process of cavernous fibrosis left the Center with noncavernous fibrosis moderately advanced. The disease of another 10 patients of the same group remained far advanced, but the cavities closed and they were included, therefore, in the noncavernous fibrosis groups. The bacteriologic condition of the 3 patients with cavernous fibrosis also improved, and they were noninfectious at the time of discharge from the Center.

It was possible to achieve these results through prolonged antimicrobial treatment (60 per cent of the trainees had never received this type of treatment prior to their admission) and the rehabilitation activities.

Failures (Not Rehabilitated)

Medical, social, and emotional problems were the causes for what failures there were, for, despite permanent alertness on the part of the

TABLE 3

MEDICAL CONDITION ON DISCHARGE COMPARED WITH STATUS ON ADMISSION

Type of Lesion	Minimal		Moderately Advanced		Far Advanced	
	Admission	Discharge	Admission	Discharge	Admission	Discharge
Infiltration without cavitation	—	—	3	—	1	—
Fibrosis with cavitation	—	—	4	1	18	3
Fibrosis without cavitation	7	7	148	159	55	66
	7	7	155	160	74	69

Center's team, it was not always possible to find the right solution for each trainee. The general reasons for failure were:

4,	10.3%,	disciplinary reasons (these trainees had spent from 5 to 10 years in hospitals)
4,	10.3%,	family reasons
3,	7.6%,	emotional reasons (these trainees were transferred to mental hospitals)
16,	41.0%,	within the probationary period (mainly because of medical reasons)
12,	30.8%,	relapses
39	100.0%	

The tuberculous lesions in the 16 failures "within the probationary period" were unstable during that period, and reactivation was noticed at the first examination for work tolerance. (Most of this occurred in 1952 before the introduction of prolonged antimicrobial treatment, and 11 of the trainees had already spent from five to ten years in hospitals.) The medical status of the 16 was: tuberculous peritonitis, 1; chronic pulmonary tuberculosis, 2; tuberculous bronchitis, 2; chronic tuberculous empyema, 2; signs of reactivation on the affected side, 5; and signs of reactivation on the other side, 4.

Of the 12 patients with "relapses," 8 suffered from tuberculous lesions of the lungs; 2, of the bones; 2 had nontuberculous complications (bronchial asthma and cystic lung). Of the 10 tuberculous relapses (3 per cent of the total number of trainees), in only 4 of the cases could it be assumed that work was the deciding factor; these occurred in 1953. It is of interest that there was only one relapse in the carpentry section, which was considered the most difficult trade.

Summary of results:

244,	81.60%,	were fully rehabilitated.
16,	5.36%,	were partly rehabilitated (these were trainees who left the Center with full working capacity but without acquiring any trade or, having acquired a trade, could not work eight hours a day because of medical or emotional reasons).
39,	13.04%,	were not rehabilitated.

"Program B"

This program was added to the general rehabilitation program to help effect the rehabilitation of patients in the last stages of medical treatment, particularly those in need of higher work-tolerance, as well as of further social and emotional readjustment. These convalescents did not participate in the vocational training, but were engaged in some type of interesting physical work according to their personal inclinations. Included was a group of housewives, mainly of Asian origin, mothers of numerous children, who had never been trained for their duties. They now received training in housekeeping under the conditions that prevailed in Israel.

During a short period of three to six months, on this program, 202 convalescents (117 men and 85 women) were rehabilitated as follows:

90,	44.5%,	returned to or were placed in jobs
12,	6.0%,	were placed in vocational training projects outside the Center
2,	1.0%,	were sent to schools to continue studying
23,	10.9%,	entered sheltered workshops
14,	7.4%,	were set up in small businesses
37,	18.3%,	returned home (among these, many housewives)
16,	8.0%,	returned to the hospital
1,	0.5%,	committed suicide
7,	3.4%,	unknown

Program B complemented the basic rehabilitation plan. It enabled the convalescents to adjust to conditions prevailing in Israel and to begin living like normal, healthy citizens.

Follow-up Studies

Only after three to six years of follow-up observation could conclusions be drawn as to results. It was during this period that the majority of the relapses occurred. This follow-up study is of special interest as it took place during a period of mass immigration and absorption, accompanied by an economic crisis. In addition, there was a serious lack of understanding of the needs of the post-tuberculous patient among the population, as shown in the following quotation from a graduate's letter:

> My difficulties, which exist even now to a certain extent, began immediately upon my

TABLE 4

PERSON-YEARS OF OBSERVATION OF TRAINEE AFTER DISCHARGE FROM THE REHABILITATION CENTER

Duration of Follow-up Study in Years	Number of Persons	Died during Period	Lost from Observation†	Middle Point of Period	Person Years
x*	lx	dx	wx		
0–1	47	1	—	0.5	47 × 0.5 = 23.5
1–2	42	—	2	1.5	41 × 1.5 = 61.5
2–4	90	1	3	3.5	88.5 × 3.5 = 309.75
4–6	81	—	6	5.5	78 × 5.5 = 429
Total	260	2	11	—	823.75

* x = duration of follow-up years; lx = number of persons in this period; dx = number of deaths; wx = number of persons lost from observation.

† For the persons lost from observation a half credit is given in the calculation of person-years.

The table shows the average person-years of observation for each person to be: 823.75:260 = 3.2 years.

Two graduates died, one of myeloid leukemia and the other of malignant growth of the testes. All contact was lost with 11 of the graduates.

> discharge from the Center. At first, many people refused to meet with me, and it was very difficult to find suitable company. While things were made easy for me at work, and I received better housing, no friend tried to help me in my efforts to adjust to everyday life.

The follow-up data were obtained through information received from the patient himself and from the chest clinic he attended for supervision. About 30 per cent of the graduates returned to the Center for medical check-ups in addition to their regular attendance at the clinic. There were a number of reunions of the graduates, the largest in 1956 when about 80 per cent of the graduates participated. Some data on this follow-up study are given in table 4.

The follow-up studies were concentrated particularly on the medical condition, especially exacerbations of the disease: 6 graduates (2 per cent) suffered relapses (5 in the lungs and one in the kidneys). These occurred between the first and third years following their discharge from the Center. Before entering the Center they had spent five to eight years in hospitals. Two of the 6 trainees had been treated by thoracoplasty before admission to the Center; 2 had lived in difficult social and emotional situations after discharge from the Center; and 2 had performed very strenuous work.

Vocational Follow-up Studies

Considering the unsteady employment conditions prevailing on the Israel labor market, the reduction of personnel (efficiency discharges and businesses closing down), and the slogan, "Last in—first out," the graduates of the Center have not done too badly.

Of the 260 patient-trainees who were completely or partially rehabilitated, 80 per cent held skilled jobs; 5 per cent other jobs; and 15 per cent were housewives, people not yet placed, or those whose whereabouts were unknown to us. It can be pointed out that many of the graduates attained good positions: one became a bank manager; another, the administrative director of a hospital; a third, a foreman, et cetera, and some even became employers of labor.

Social Follow-up Studies

One purpose of the Center was to make taxpayers of people who had been supported by social agencies. The follow-up studies showed that 85 per cent of the graduates are working, and 63 per cent of these pay taxes while the others are exempt either because of the large families they support or the low salaries they receive.

The general improvement in their condition encouraged many of the graduates to establish families: 68 per cent are married now compared with only 8 per cent of the admissions to the Center. Many support parents and other relatives. One-third of the graduates, who upon discharge from the Center did not have proper homes, have in the meantime acquired suitable housing, and some have even moved to much better apartments than those originally provided for them.

Of particular interest is the follow-up observation on the 39 patients who were not rehabilitated: 8 are still in hospital; 13 are not working or are employed on government work projects; 10 are working; 3 left the country; and the whereabouts of the remaining 5 are unknown.

SUMMARY

Shortly after the establishment of the state of Israel, the Rehabilitation Center for Tuberculous

Patients was opened at Neve Chaim, Israel, as one of the many forms of rehabilitation being introduced into the country at the time. The 501 tuberculous patients sent to the Center for rehabilitation came from the vast group of immigrants who had come to Israel during its first years of statehood. The treatment of the patients had been prolonged from the clinical point of view, and their conditions had been made even more complicated as a result of their difficult psycho-social problems.

The main aim of the Center was to restore the patient's self-respect and make him a taxpaying citizen through environmental treatment, individual approach, and vocational training. Of course, when necessary, the medical treatment was continued to ensure the patient's eventual discharge. The follow-up observations, which ran for more than six years, showed that 80 per cent of the patients succeeded in becoming absorbed into their communities as normal, healthy citizens.

What was done at the Center in a preparatory way should be done today within the framework of hospitals and outpatient clinics. Thus, the patient could be rehabilitated within a much shorter period and returned to the community before too much time had elapsed.

While today there is much greater understanding among doctors of the patient's problems, there is still a serious need for a widespread public information campaign, for in the long run it is the public who will guarantee the success of these efforts.

Sumario

La Rehabilitación de los Tuberculosos en Israel

Poco después del establecimiento del estado de Israel, se inauguró el Centro de Rehabilitación para Tuberculosos en Neve Chaim, Israel, como una de las muchas formas de rehabilitación introducidas en el país para dicha fecha. Los 501 tuberculosos enviados al Centro para rehabilitación procedían del inmenso grupo de inmigrantes que habían ido a Israel en los primeros años de su creación como estado. El tratamiento de los enfermos había sido prolongado desde el punto de vista clínico y se había complicado aun más la situación de los mismos a consecuencia de sus difíciles problemas psico-sociales.

La principal meta del Centro era devolver al enfermo su dignidad y convertirlo en ciudadano contribuyente al Erario Público por medio de atención al medio ambiente, iniciación de relaciones personales y preparación profesional. Por supuesto, cuando era necesario, se continuaba el tratamiento médico para garantizar en su oportunidad el alta del enfermo. Las observaciones subsiguientes durante más de seis años revelaron que 80 por ciento de los sujetos lograron ser absorbidos en sus respectivas colectividades como ciudadanos sanos y normales.

Lo hecho en el Centro en forma preliminar debe ser llevado a cabo actualmente al amparo de hospitales y clínicas para enfermos ambulantes. De ese modo, podría rehabilitarse al enfermo en un período mucho más breve y devolverlo a la colectividad antes de transcurrir demasiado tiempo. Aunque existe hoy día una comprensión mucho mayor de parte de los médicos acerca de los problemas de los enfermos, se necesita todavía con urgencia una campaña general de información al público, pues al fin y al cabo el público es el que garantiza el éxito de tales esfuerzos.

Resume

Réadaptation du tuberculeux en Israël

Peu de temps après la création de l'état d'Israël et parmi de nombreux projets de réhabilitation établis dans le pays, un centre de réadaptation pour tuberculeux fut ouvert à Neve Chaim, Israël. Les 501 tuberculeux envoyés à ce Centre de réadaptation faisaient partie de la grande masse d'immigrants venus à Israël pendant les premières années de sa création. Du point de vue clinique ces malades avaient été traités pendant une longue période et leur état était d'autant plus compliqué qu'ils présentaient tous des problèmes psychosociaux complexes.

Le but principal du centre était de permettre au malade de retrouver sa dignité et de redevenir un citoyen normal grâce à une éducation professionnelle, un traitement individuel et un réajustement au milieu. Bien entendu, le traitement médical, lorsqu'il était nécessaire, fut continué pour permettre au malade de quitter le centre, rétabli. Les malades ont été suivis pendant plus de six ans et 80% d'entre eux ont réussi à redevenir des citoyens normaux et ont été absorbés par leurs communautés.

Ce qui a été fait dans le centre en guise de préparation, devrait être accompli normalement dans les hôpitaux et les dispensaires. De cette façon le malade pourrait être réadapté beaucoup plus rapidement et réintégré plus tôt dans sa communauté. Bien qu'aujourd'hui les médecins soient plus conscients des problèmes de leurs malades qu'autrefois, il est nécessaire de continuer une vaste campagne d'information du public car c'est

ce dernier qui à la longue sera la garantie du succès de ces efforts.

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ENVIRONMENTAL VERSUS CONSTITUTIONAL FACTORS IN THE DEVELOPMENT OF TUBERCULOSIS AMONG NEGROES[1,2]

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Introduction

Although tuberculosis morbidity and mortality are higher among Negroes than among whites, there is no unanimity of opinion as to whether this is due to environmental factors or to a constitutionally lower resistance of the Negro to this disease.

Pinner and Kasper (1) found marked differences at autopsy in the response to tuberculosis of adult Negroes and whites, indicating a lower resistance in the Negro. The differences consisted of a greater frequency in the Negro of hematogenous and lymphogenous spread of the tuberculous disease, of massive caseation of lymph nodes, and of exudative lesions which do not respect normal anatomic boundaries of organs. Rich (2), also on the basis of autopsy findings, reports that "there is definite clinical and anatomical evidence that the Negro very commonly reacts to the tubercle bacillus in a manner which is different from that characteristic of the white race." The clinical and roentgenographic studies of Israel and Payne (3) bear out the necropsy studies in that they found a "group difference in the characteristics of tuberculosis among whites and Negroes," but they state that "anatomically typical childhood-type tuberculosis is apparently rare in white adults and infrequent in adult Negroes."

These reports leave little doubt that there is a difference in the type of response of the two races to infection with tubercle bacilli. They do not, however, indicate the relative importance of this difference in the total picture of tuberculosis morbidity and mortality, nor do they offer an explanation for it. Pinner and Kasper recognize this in their warning that their study was based upon autopsy material only, and that "conclusions drawn from them can be applied to the much greater number of nonfatal cases only with the utmost caution and probably not without modifications." They are of the opinion that the difference in reaction is a racial characteristic, but admit that "the hypothesis of genotypic differences recommends itself strongly because of the apparent impossibility to explain the matter completely by any other alternative." "But," they add, "this process of exclusion does not constitute proof."

Evidence in favor of environmental causes for the excess tuberculosis morbidity and mortality in the Negro is presented by Bushnell (4), who points out that from 1822 to 1848 in Charleston, South Carolina, the tuberculosis mortality was about the same for whites and Negroes, which he attributes to the fact that, in general, masters provided food, shelter, and some degree of medical care for their slaves. After the Civil War, however, "when their emancipation thrust them unprepared into the struggle for existence their sufferings and errors are revealed in an enormously increased mortality," the rates among Negroes became two or three times as high as among whites.

That there is a relationship between the development of tuberculosis and such nonconstitutional factors as economic level, housing, conditions of exposure, type and conditions of employment is generally accepted. When, however, race is added to these factors, the relative importance of environmental and constitutional factors becomes difficult to determine. That the Negro generally lives under poorer environmental conditions is well known. The question is whether these lower standards of living are sufficient in themselves to explain his higher tuberculosis morbidity and mortality.

If the races could be placed in an identical environment, any differences in reaction to tuberculosis might be ascribed to factors of a constitutional nature. Such an identical environment for whites and Negroes is found in the hospitals of the New York State Department of

[1] From the New York State Department of Health, Division of Tuberculosis Control, Albany, New York.

[2] Presented in part before the Medical Session, as part of *Section 2C*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

Mental Hygiene. Patients in these hospitals are fed, housed, and cared for without regard to race, so that the environment is the same for both, and any differences in tuberculosis morbidity and mortality may, therefore, be due to constitutional factors. On the other hand, under these conditions, a close similarity in tuberculosis morbidity and mortality may be interpreted as evidence of the importance of nonconstitutional factors.[3]

Although the term "identical" is used with reference to the mental hospital environment, there probably are some slight differences in environment for the two races. For example, in some hospitals the proportion of Negroes is higher than in others, so that the proportion of Negro patients may be higher in some wards; or there may be more overcrowding of some wards than others. In general, however, the term "identical" is used to indicate the absence of any factors which specifically favor patients of one race over the other. Certainly, those slight differences in environment for the races which may exist within these hospitals cannot be compared in magnitude with those found in the communities from which these patients come—New York City, for example.

Materials and Methods

The data used in this report were obtained since 1941 during the conduct of a tuberculosis control program among patients and employees in the institutions of the New York State Department of Mental Hygiene. In this program all patients are examined roentgenographically upon admission and surveyed routinely thereafter. Up to this writing, data are available for five routine chest roentgenographic surveys of all patients, so that the incidence of tuberculosis can be calculated and the rates compared.

All cases of tuberculosis included in this report were newly developed within the institutions. In every instance there was at least one (and often more than one) chest roentgenogram which showed the absence of pulmonary disease. The presence of any roentgenographic evidence of pulmonary disease at the time of admission to the hospital, whether this was considered tuberculous in origin or not, was sufficient to disqualify the case from this study. Due to the difficulty of obtaining adequate studies of sputum or gastric contents, laboratory confirmation of the diagnosis in some patients was lacking, but the duration of the clinical and roentgenographic follow-up observation of these patients was long enough to make the diagnosis reasonably certain.

The average length of stay of patients within the hospitals is about six years. Although white patients spent slightly more time in these hospitals than nonwhites (six years as compared with five and one-half years), the length of stay was sufficiently long for both races to permit a study of the effect of similarity of environment upon tuberculosis morbidity and mortality.

Patients with active tuberculosis are segregated and treated in special tuberculosis centers. Patients from all institutions who are found with active disease, either upon admission or subsequently, are transferred to these centers, where they are treated until the disease becomes inactive. In this way exposure to infectious tuberculosis of patients in the general wards of the hospitals is kept at a minimum, and is no different for whites than for Negroes.

The term "nonwhite" used in this report refers mainly to Negroes, since the number of patients other than white or Negro is small.

The terms "nonconstitutional" and "environmental" are used interchangeably to indicate such factors as housing conditions, economic level, conditions of exposure to tuberculosis, type and conditions of employment, and others which are often included under the general term "standard of living."

Results

Incidence of Tuberculosis

Before the rate of development of tuberculosis among hospitalized mental patients is considered, the incidence of the disease in the general population must be known, so that a basis for comparison may be established. Unfortunately, such data are not available so that only estimates can be used, based upon the number of new cases reported annually. Admittedly, not all cases of tuberculosis are reported, nor do all those patients reported in a given year develop their disease during that year. Also it is recognized that case-finding activities are greater among nonwhite populations than among whites. Nevertheless, in the absence of more accurate data, it seems reasonable, for the purpose of this report, to use rates of newly reported cases of tuberculosis as approximations of the incidence rates in the two races. Data from New York City are used because that city contains the largest segment of nonwhite population in the state.

[3] There are no data available regarding the prevalence of infection in the two races on admission; however, that there is probably no significant difference is suggested by the fact that, among admissions to the Willowbrook State School for mental defectives, with a younger population drawn from New York City, the percentage of reactors to intermediate-strength PPD among whites was 13.4 and among nonwhites it was 12.2.

Incidence of tuberculosis in New York City: In New York City from 1943 through 1955, the time period similar to that used in this study of mental hospital patients, the tuberculosis case rates were about four times as high for Negroes as for whites (table 1). During this period, the rate of decline was roughly parallel for the two races, and the ratio of Negro to white incidence remained practically unchanged at about 4 to 1.

Incidence of tuberculosis in mental hospitals: In contrast to this marked difference in incidence between the races in New York City, there was a relatively small difference among hospital patients. In the earlier period of this study, the incidence among Negroes was slightly more than six per 1,000 person-years, compared with four among whites, a difference of about 50 per cent. In the latter part of the observation period, the rate for Negroes had decreased to 2.7 and for whites to almost 2.1, a difference of only 30 per cent (table 2, figure 1). The rate of decrease among Negroes was 56 per cent; among whites, 49 per cent. Thus, the incidence fell somewhat more rapidly among Negroes than among whites.

Even though the rate of decrease among Negro patients was at least as rapid as among whites, the difference in the levels of the incidence rate for the two races is to be explained. This difference was partly due to differences in the age distribution of the two races. As is true in the general population, Negroes in mental hospitals are younger than whites. Since the incidence of tuberculosis is somewhat higher among younger patients (less than forty-five years of age), the rates were adjusted to compensate for this age difference. With this adjustment the differences in rates were reduced so that in the earlier part of the study there was an excess of 27 per cent in incidence among Negroes, while, at the end, the excess was only 8 per cent (table

TABLE 1

CASES REPORTED IN NEW YORK CITY PER 100,000 POPULATION, BY RACE*

Year	Whites	Negroes
1943	81	270
1944	71	262
1945	63	249
1946	64	252
1947	68	254
1948	72	281
1949	70	302
1950	65	256
1951	63	263
1952	58	253
1953	59	259
1954	51	223
1955	49	209

* Based upon data from reports of the New York Tuberculosis and Health Association.

TABLE 2

CASES AND CRUDE AND AGE-ADJUSTED INCIDENCE RATES FOR TUBERCULOUS PATIENTS IN MENTAL HOSPITALS IN THE FIRST–THIRD SURVEY PERIOD AND THIRD–FIFTH SURVEY PERIOD BY RACE AND LENGTH OF STAY, AND RATIO OF NONWHITE TO WHITE RATES

Survey Period and Race	Length of Stay Period (Years)								
	Total			Less than Two Years			Two Years or More		
	Cases	Rate per 1,000 Person-Years		Cases	Rate per 1,000 Person-Years		Cases	Rate per 1,000 Person-Years	
		Crude Rate	Age-adjusted Rate		Crude Rate	Age-adjusted Rate		Crude Rate	Age-adjusted Rate
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
First–third survey									
White	1,909	4.09	4.12	162	1.74	1.74	1,747	4.67	4.71
Nonwhite	274	6.09	5.22	57	4.68	4.27	217	6.62	5.59
Ratio $\frac{\text{Nonwhite}}{\text{White}}$		1.49	1.27		2.69	2.45		1.42	1.19
Third–fifth survey									
White	631	2.09	2.10	73	1.22	1.21	558	2.30	2.32
Nonwhite	95	2.70	2.26	23	2.42	2.30	72	2.80	2.24
Ratio $\frac{\text{Nonwhite}}{\text{White}}$		1.29	1.08		1.98	1.90		1.22	0.97

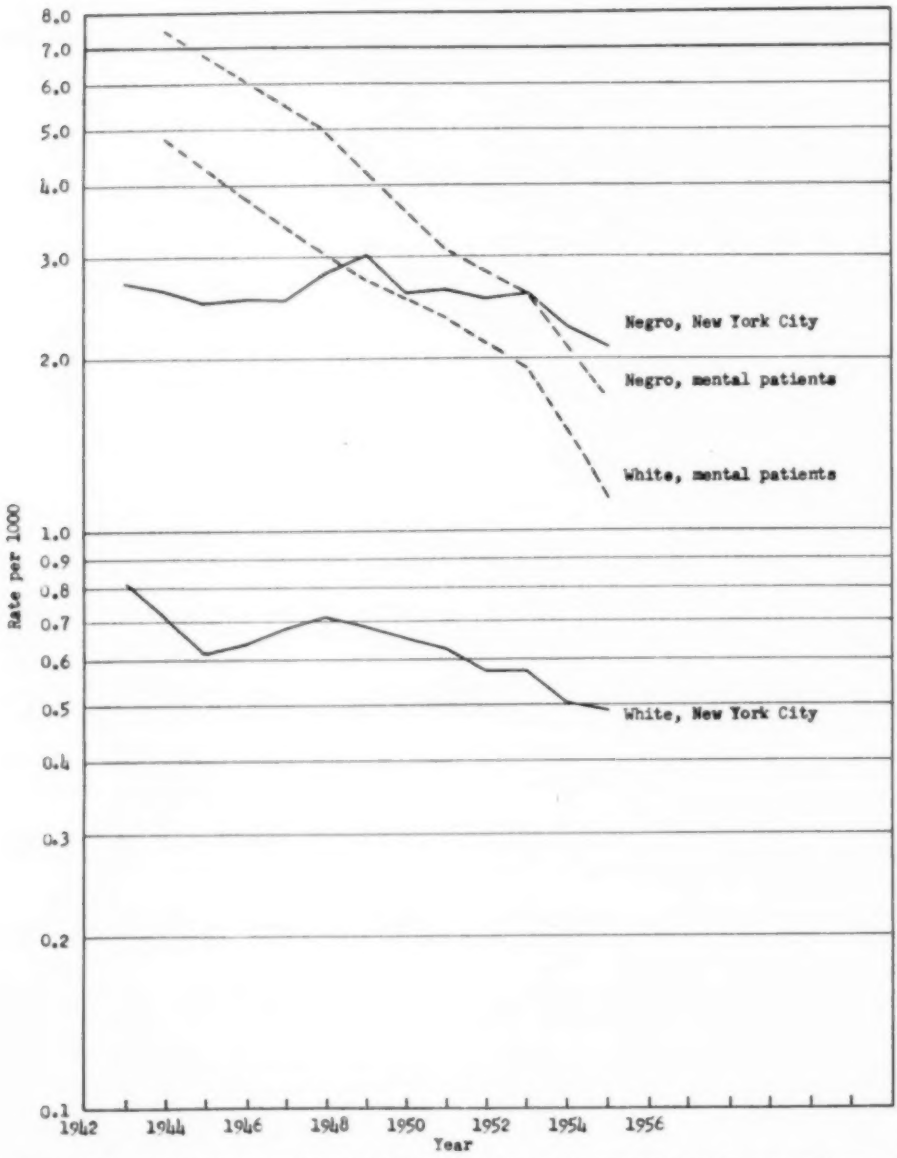

FIG. 1. Tuberculosis case-reporting rates in New York City and tuberculosis-incidence rates in mental hospitals, by race.

2, column 3). A similar adjustment for age differences in the New York City population would probably also lower the incidence rate among nonwhites as compared with whites, but the difference would not materially affect the Negro to white ratio of 4 to 1. It is, therefore, apparent that, when environmental conditions become similar for the two races, the rates of developing tuberculosis tend to become similar.

In addition to the rate at which tuberculosis

is developed, the stage of disease at the time of diagnosis is of importance because of the close correlation between stage and prognosis. Fewer Negro patients were found with minimal, and more with far advanced, disease than among whites; but the differences decreased with time. Thus, between 1942 and 1949 Negroes developed about half as much minimal, and twice as much far advanced, disease as whites. Between 1951 and 1957, although the proportion of minimal cases increased and of far advanced cases decreased for both races, the improvement was more pronounced among the Negroes, so that the proportion of minimal cases among Negroes had increased to 77 per cent of that of whites, and the excess of far advanced cases among Negroes was only 60 per cent higher than among whites (table 3). In fact, within these fifteen years, the distribution of disease by stage had so improved for Negro patients that the figures for nonwhites in the later period were essentially the same as for white patients in the earlier period.

There also was a relationship between the incidence rates within the institutions and the stage of disease on diagnosis. In half of the mental hospitals with higher incidence rates, white patients had proportionately more minimal, and less far advanced, disease than Negroes, while in the hospitals with lower rates Negroes had almost the same proportion of minimal, and fewer far advanced, cases than whites (table 4). Moreover, in New York City, in spite of the great difference in the incidence of tuberculosis in the two races, the distribution by stage of disease of newly reported cases is about the same for Negroes as for whites (5).

Thus, while the data regarding stage of disease on diagnosis do not give as definite a picture of the relative importance of environment and constitution as in the case of incidence rates, they do suggest that environmental factors probably play a predominant part.

TABLE 3

NEW CASES OF TUBERCULOSIS: STAGE OF DISEASE ON DIAGNOSIS

(Rate per 100)

Stage	1942–1949		1951–1957	
	White	Nonwhite	White	Nonwhite
All stages	100.0	100.0	100.0	100.0
Minimal	49.3	23.7	54.3	41.9
Moderately advanced	23.8	24.1	27.1	28.4
Far advanced	26.8	52.5	18.6	29.7

TABLE 4

STAGE OF DISEASE ON DIAGNOSIS IN HOSPITALS OF NEW YORK STATE DEPARTMENT OF MENTAL HYGIENE

Stage of Disease	White Rate per 100	Nonwhite Rate per 100
Hospitals with Higher Incidence Rates*		
All stages	100.0	100.0
Minimal	54.3	37.3
Moderately advanced	28.4	21.6
Far advanced	17.4	41.2
Hospitals with Lower Incidence Rates†		
All stages	100.0	100.0
Minimal	54.4	52.2
Moderately advanced	23.7	43.5
Far advanced	21.9	4.3

* Incidence rates ranged from 2.9 to 6.9 per 1,000 person-years.

† Incidence rates ranged from 0.3 to 2.4 per 1,000 person-years.

Although the conduct of a tuberculosis control program in a "captive" group, such as hospitalized mental patients, is simplified because of the relative ease with which they can be periodically surveyed and cases of tuberculosis segregated, it suffers from the disadvantage that, when a case of tuberculosis develops in the interval between surveys, the disease can spread rather rapidly to other patients because of the close contact between patients required by institutional life. As a result, in spite of the marked decrease in the incidence of tuberculosis resulting from the program, the rate among hospitalized mental patients remains higher than among white residents of New York City, although it is lower than among Negroes in the City (figure 1). Because of this, admission to the hospitals had a different effect upon the races.

The white patient entered the hospitals from an environment in which the incidence of tuberculosis, as measured by case rates, was much lower than that in the hospitals, so that his chances of developing tuberculosis became greater than they were in his home environment. The Negro, on the other hand, entered an environment in which the hazard of tuberculosis was more nearly similar to that of his usual place of residence. As a result, the incidence of tubercu-

losis increased considerably among whites during their hospital stay, while the the rates among Negroes showed no material change. It is obvious that a change in environment is not *immediately* accompanied by a corresponding change in the incidence of tuberculosis, since the impact of the original environmental factors takes some time to subside, just as it takes time for the new environment to take full effect. This is shown in table 2 (columns 6 and 9), in which the length of stay is divided into periods of less than two years and two years or more.

The table shows that, even in the period of lower risk (between the third and fifth surveys), the age-adjusted rate among white patients increased with the duration of hospital stay from 1.2 among those resident less than two years to 2.3 among those hospitalized two years or more. Among nonwhites, for whom the hospital environment presented less of a change in the hazard of developing tuberculosis, the rate during the first two years of hospital stay, 2.3, did not change materially during longer hospitalization, 2.2.

It is concluded, therefore, that under similar conditions, the Negro is probably not much more prone to develop tuberculosis than the white man. The obvious question to be answered is, what are his relative chances of survival when he does develop the disease?

TABLE 5

SURVIVAL OF WHITE AND NONWHITE PATIENTS WHO DEVELOPED TUBERCULOSIS IN NEW YORK STATE MENTAL HOSPITALS, 1941–1949

Period of Observation	Survival—Rate per 100 Stage of Disease					
	Minimal		Moderately Advanced		Far Advanced	
	White	Non-white	White	Non-white	White	Non white
Years						
Males						
0–1	94.2	89.2	76.1	62.8	30.3	10.4
1–2	89.1	80.0	65.2	49.2	17.4	5.9
2–3	85.7	76.4	56.9	45.7	12.0	5.9
3–4	81.4	76.4	54.1	45.7	9.9	—
4–5	79.0	76.4	49.6	39.2	7.4	—
Females						
0–1	92.3	95.2	71.2	62.5	21.9	6.6
1–2	87.6	74.1	61.8	44.6	11.3	6.6
2–3	81.7	68.8	56.9	44.6	8.9	6.6
3–4	79.5	62.5	53.0	29.7	5.9	6.6
4–5	74.1	62.5	50.1	29.7	5.9	6.6
Number of Patients at Beginning of Study						
Males	531	37	243	43	287	67
Females	260	21	139	16	146	61

TABLE 6

EFFECT OF ANTIMICROBIAL THERAPY UPON THE SURVIVAL OF TUBERCULOUS PATIENTS
Pilgrim State Hospital: 1950–1955

Period after Beginning Drug Therapy	Survival—Rate per 100 Stage of Disease					
	Minimal		Moderately Advanced		Far Advanced	
	White Patients	Negro	White Patients	Negro	White Patients	Negro
Years						
0–1	95.5	89.7	96.3	84.9	83.4	77.9
1–2	90.3	87.7	89.8	78.0	74.1	75.3
2–3	86.3	87.7	86.1	76.8	69.7	69.7
3–4	86.3	87.7	82.4	67.2	65.6	65.9
4–5	86.3	87.7	82.4	67.2	63.4	65.9
5–6	86.3	87.7	82.4	67.2	63.4	65.9
Number of Patients at Beginning of Study						
	226	60	437	115	340	142

Survival from Tuberculosis

If the Negro is constitutionally less resistant to tuberculosis, this should become apparent from a study of patients whose disease was detected soon after its development and kept under long periods of observation, so that survival rates might be measured. This was done for a group of 1,606 white and 245 Negro patients who developed tuberculosis between 1941 and 1949 while they were residents in state mental hospitals. During most of this period antimicrobial therapy was not available, and during the last two years it was used to a very slight extent.

The survival of nonwhite patients was somewhat lower than of whites (table 5). At least part of the difference was probably due to the fact that the rates were based upon the total number of deaths among tuberculous patients, whether they were due to tuberculosis or not. Since the mortality from all causes is higher

among nonwhites, more nontuberculous deaths were probably included among Negroes than among whites, resulting in a reduction in the Negro survival rates. Be that as it may, the differences in survival rates of the two races are relatively small, so that racial differences probably play a minor part in the survival of tuberculous patients.

Additional evidence of this opinion is provided by a study of the effect of antimicrobial therapy upon the survival of tuberculous patients. The survival of a group of 1,003 white and 317 nonwhite tuberculous patients who received drug therapy was studied between 1950 and 1955. At the end of the six-year period of observation, no significant difference was observed between the survival of white and nonwhite patients treated (table 6). From this it is concluded that any inherent lack of resistance by the Negro, if it does exist, is slight enough to be overcome by drug therapy.

A further suggestion of the minor role played by racial differences in survival from tuberculosis is provided by a study of case fatality ratios. These relate the proportion of cases reported during any year to the deaths during the same year, and provide an approximation of the ability of the population to survive the development of tuberculosis. If the ratio of cases to deaths is high, it indicates a high survival rate; if low, a low rate.

For every year from 1951 through 1957 the ratio of cases to deaths in New York City was higher among Negroes than among whites. In other words, during these years more cases of tuberculosis for each death were reported among Negroes than among whites (table 7). While these higher ratios among Negroes are probably partly due to more extensive case-finding efforts among this group, they also suggest that the ability of Negroes to survive tuberculous disease is similar to that of whites.

TABLE 7

RATIOS OF REPORTED CASES TO DEATHS FOR WHITES AND NEGROES

New York City, 1951–1957

Year	Ratios of Cases to Deaths	
	Whites	Negroes
1951	3.04	3.49
1952	3.79	3.92
1953	4.67	5.95
1954	4.49	6.60
1955	4.44	6.49
1956	4.71	6.62
1957	4.81	6.73

Data from: Tuberculosis in New York City 1957, Lowell, A. M., New York Tuberculosis and Health Association.

DISCUSSION

In considering the results of this study, it is important first to point out that there is nothing inherent in mental disease which makes the reaction to tuberculosis of mental patients different from that of mentally sound persons. In previous studies it has been shown that differences in tuberculosis morbidity and mortality between hospitalized mental patients and the general population may be explained by differences in factors of epidemiologic nature in the two groups (6, 7). It is, therefore, believed that conclusions drawn from this study of mental patients can be applied to the population as a whole.

The data show that within the mental hospitals the tuberculosis morbidity decreased at about the same rate for white and nonwhite patients, and this is attributed to the similar environment for the races. It may be seen from figure 1, however, that this also holds true for New York City, where environmental conditions are much different for the races, so that these findings may appear contradictory. This may be explained by the fact that, while the difference in the *level* of tuberculosis is an indication of the effect of environment upon the races, the similarity in the *changes* in incidence rates suggests that both races benefit from any important improvement in living conditions. In other words, whatever the changes may have been in conditions in New York City which resulted in a variation in tuberculosis morbidity among whites have also affected the nonwhites in a similar direction. Whether or not these changes are due to variations in the general prosperity and standard of living in New York City since 1943 cannot be shown, but it is quite probable that this is one of the important reasons for the changing incidence of tuberculosis in the City.

The data also show that the marked decrease in the incidence of tuberculosis among hospitalized patients has been practically the same for both races. This in itself is strongly suggestive of the importance of environmental factors, since

it is hardly probable that the decrease was due to changes in environmental factors among whites and to constitutional factors among Negroes.

From the available data, the relative importance of constitutional factors in determining the stage of disease on diagnosis is not as clear-cut as that for incidence, survival, and response to drug therapy. The fewer minimal and the larger number of far advanced cases among Negroes at the time of diagnosis suggest a racial influence. On the other hand, the greater increase in the proportion of minimal cases and the decrease in far advanced cases among Negroes during the period of the study suggest an environmental causation. The inverse relationship between minimal disease in Negroes and higher incidence of tuberculosis within the hospitals is probably also due to environmental factors, although the nature of these is not readily apparent. The close similarity between the races in distribution by stage on diagnosis in New York City is important evidence against the significance of racial factors. On the whole, it appears that, while both constitution and environment may affect the stage of disease on diagnosis, the data point to the latter as probably of greater significance.

Although the evidence presented suggests that racial factors do not play a major part in the tuberculosis morbidity and mortality of the Negro, it does not offer any explanation for the differences in the anatomic reaction to the disease found by Rich and others. There is no doubt that the Negro may show autopsy findings indicative of a lower resistance to tuberculosis, but the importance of this depends upon its relative frequency. Neither Rich nor Pinner and Kasper present any quantitative data to indicate the frequency of this type of response in the total picture of the natural history of tuberculosis in both races. Pathologists are hardly in a position to do so; yet, in order to properly evaluate their findings, such a study is essential.

Summary

A group of white and Negro patients, living in identical environments (mental hospitals of New York State) were studied to determine the effect of environment upon the morbidity and mortality from tuberculosis. In factors of importance in the reaction to tuberculosis, the races showed no significant differences. Within a similar environment both races developed the disease at the same rate. Among those who developed tuberculosis, the proportion who died of the disease was similar for both races. Antimicrobial drugs benefited both races equally. Because of these findings, it is concluded that, under similar conditions of environment, the response of the Negro to tuberculosis is similar to that of the white, and that racial factors probably play a minor role in producing differences in tuberculosis morbidity and mortality.

Sumario

Los Factores del Medio Ambiente Comparados con los Constitucionales en la Producción de Tuberculosis en los Sujetos de Raza Negra

Este e studio comprendió un grupo de sujetos de las razas blanca y negra, que vivían en medios idénticos (frenocomios del Estado de Nueva York) con mira a determinar el efecto del ambiente sobre la morbidad y la mortalidad tuberculosas. Entre los factores de importancia en la reacción a la tuberculosis, las razas no mostraron diferencias significativas. En un ambiente semejante, ambas razas contrajeron la dolencia en la misma forma. Entre los que la contrajeron, la proporción que falleció de la enfermedad fué similar en ambas razas. Los antimicrobianos beneficiaron por igual a ambas. Debido a estos hallazgos, se deduce que, en condiciones semejantes del medio, la respuesta del sujeto de raza negra a la tuberculosis es semejante a la del blanco, y que los factores étnicos desempeñan probablemente un papel secundario en la producción de diferencias en la morbidad y la mortalidad tuberculosas.

Resume

Comparaison des facteurs constitutionnels et des conditions du milieu dans l'apparition de la tuberculose chez les nègres

Les auteurs ont étudié un groupe de malades blancs et nègres vivant dans des conditions identiques (hôpitaux psychiatriques de l'État de New York) afin de déterminer les effets du milieu sur la morbidité et la mortalité tuberculeuses. Ils n'ont pas trouvé de différence entre les deux races en ce qui concerne les facteurs importants dans la réaction à la tuberculose. En milieu identique, la maladie affecte la même proportion d'individus dans les deux races. Les médicaments antimicrobiens donnent les mêmes résultats dans les deux groupes. Ces résultats permettent aux auteurs de conclure que, placé dans des conditions de milieu identique, le nègre réagit à la tuberculose comme le blanc et que le facteur racial joue un rôle négli-

geable dans les différences de morbidité et de mortalité tuberculeuses.

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BRONCHOGRAPHIC PATTERNS FOLLOWING RESECTION FOR PULMONARY TUBERCULOSIS[1]

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Introduction

Bronchographic studies in patients who had undergone pulmonary resection have disclosed valuable information regarding the expansion patterns of remaining parenchyma, the defects of bronchial stumps, and the frequency of postoperative bronchiectasis (1–4). The present observations and comments are based upon a review of 52 post-resection bronchograms taken during the years 1954–1958 at Glen Lake Sanatorium. The study was prompted by certain features of this series of cases: All of the 52 subjects had surgery as part of treatment for tuberculosis; a variety of resection procedures, either single or combined, was employed (tables 1, 2); and, finally, preoperative bronchograms were available for comparison in the majority of cases.

The indications for the postoperative bronchography were suspected bronchiectasis or bronchopleural fistula in 23 instances and "routine investigation" in the remaining 29. Neither group consisted of consecutive cases. The interval between resection and bronchography ranged between three to six months (28 cases) and seven and one-half years (one case). With few exceptions, aqueous propyliodine (Dionosil®) was used as the contrast medium.

Observations

Expansion Patterns

The usual postoperative patterns are diagrammatically illustrated in figures 1–20. (All of the figures may be found at the end of the article.) They are, by and large, in agreement with those described by Ettinger and associates (1), Wallace and Pillman (2), Crellin and associates (3), Stutz and Vieten (4), and Ware and Stauss (5). It is apparent that, in order to fill the space created by excision of pulmonary tissue, the remaining segments and lobes undergo a process of displacement and/or distention. The former is bronchographically demonstrated by positional shift or distortion of the corresponding bronchi, and the latter by the fanning out of the segmental branches and their tributaries. These findings are usually best seen on the lateral views. The degree of change varies directly with the volume of lung removed. Thus, little or no effect is produced by wedge resections or subsegmentectomies (figures 2, 3), while pronounced architectural alterations result from lobectomies unaccompanied by space-reducing procedures (figures 10, 12, 17, 19). Similar pattern changes, although to a lesser extent, may be caused by the volume reduction due to atelectasis or fibrotic contraction of a segment or lobe (1) (figure 14).

The portions of lung anterior to the hilum, i.e., the anterior segment, middle lobe (or lingula), and anterior and medial basal segments, appear to play a more active role in the space-filling process than the dorsal members. The right middle lobe and the lingula of the left upper lobe are particularly noteworthy in this regard and often become the major participants in post-resection events by virtue of their location and mobility (figures 6, 8, 10, 12, 19).

The upward displacement of the middle lobe after removal or shrinkage of the right upper lobe leads to a ventro-cephalad angulation of its bronchus (figures 10, 12). A dorso-cephalad elevation of the main-stem bronchus occurs under corresponding circumstances on the left side (figures 17, 21A). The drainage from these bronchi becomes impaired, resulting in retention of contrast medium or sputum in the lumen. Extreme bronchial angulation also obstructs air flow, and distressing symptoms may ensue (6). Thoracoplasty may, to various degrees, forestall or correct the abnormal angulation or curvature (figures 11, 13, 21) (6).

Thoracoplasty in conjunction with resection was performed in 14 cases (preliminary in 6, concomitant in 7, subsequent in one). It is effective in preventing or minimizing overexpansion and distortion of the remainder of the

[1] From Glen Lake Sanatorium, Oak Terrace, Minnesota.

TABLE 1

RESECTIONS PERFORMED ON THE RIGHT LUNG

Operation	Number of Cases
A. Right upper lobe:	
Wedge resection, B1-2*	1
Subsegmentectomy, B2	2
Clamp excision, B3 and adjoining portions of B-1-2-6	1
Segmentectomy, B2	1
Segmentectomy, B2, and wedge resection, B3	1
Segmentectomy, B3, subsegmentectomy, B2 and wedge resection, B1	1
Segmentectomy, B-1-2	4
Segmentectomy, B1-2, with thoracoplasty	1
Clamp excision, B1-2-6	2
Lobectomy, right upper lobe	3
Lobectomy, right upper lobe, with thoracoplasty	1
Lobectomy, right upper lobe, and wedge resection, right middle lobe	1
Lobectomy, right upper lobe, and wedge resection, right middle lobe, with thoracoplasty	1
Lobectomy, right upper lobe, and wedge resection, B6	1
Lobectomy, right upper lobe, and wedge resection, B6, with thoracoplasty	1
Lobectomy, right upper lobe, and segmentectomy, B6	2
B. Right middle lobe:	
Lobectomy, right middle lobe, and wedge resection, B1	1
C. Right upper lobe and right middle lobe:	
Bilobectomy, right upper lobe and right middle lobe, with thoracoplasty	1
D. Right lower lobe:	
Wedge resection, B6	1
Segmentectomy, B7-8	1
Lobectomy, right lower lobe	1
E. Right middle lobe and right lower lobe:	
Bilobectomy, right middle lobe and right lower lobe, with phrenic nerve interruption and thoracoplasty	1
Total	30

* B = bronchopulmonary segment: 1—apical; 2—posterior; 3—anterior; 6—superior; 7—medial basal; 8—anterior basal.

lung (figures 7, 11, 13, 16, 18, 20). Stretching of the pulmonary parenchyma is generally thought to be a cause of reactivation of tuberculosis. From this standpoint the writers are inclined to agree with Wallace and Pillman (2) that thoracoplasty or other space-filling methods should be considered in those cases "where there is any suspicion of residual reversible lesion in the segments of the remaining lung most likely to become over-distended" (2). The following case illustrates this point:

M. F. (No. 12847) entered Glen Lake Sanatorium on April 24, 1956, with bilateral far advanced pulmonary tuberculosis (figure 22*A*). Symptomatic improvement, roentgenographic clearing (figure 22*B*), and sputum conversion occurred promptly under therapy with isoniazid-PAS. The patient, on March 7, 1957, underwent resection on the right side. Although all lobes of the right lung were involved at the time of admission, by the time of surgery the only palpable disease was found in the upper lobe and the superior segment of the

TABLE 2

RESECTIONS PERFORMED ON THE LEFT LUNG

Operation	Number of Cases
A. Left upper lobe:	
Wedge resection, B1*	2
Wedge resection, B1-2	2
Clamp excision, part of B2	1
Subsegmentectomy, B1, and wedge resection, B6	1
Segmentectomy, B1, and subsegmentectomy, B2	1
Segmentectomy, B1-2	3
Segmentectomy, B1-2, with thoracoplasty	1
Clamp excision, B1-2	1
Segmentectomy, B1-2-3, with thoracoplasty	2
Clamp excision, B1-2-6, with thoracoplasty	1
Segmentectomy, B4-5-6, and wedge resection, B1-2	1
Segmentectomy, B1-2-6-9, and subsegmentectomy, B3, with thoracoplasty	1
Lobectomy, left upper lobe	1
Lobectomy, left upper lobe, with thoracoplasty	1
Lobectomy, left upper lobe, and wedge resection B6, with thoracoplasty	2
B. Left lower lobe:	
Subsegmentectomy, B6	1
Total	22

* B = bronchopulmonary segment: 1-2—apical-posterior; 3—anterior; 4—superior (lingula); 5— inferior (lingula); 6—superior (lower lobe); 9—lateral basal

lower lobe. Lobectomy and segmentectomy were accordingly carried out. Postoperative bronchograms disclosed marked expansion and upward shift of the middle lobe (figure 12). The patient left the hospital against advice on July 31, 1957 (figure 22C) and continued to take antimicrobial drugs. He returned four months later with sputum positive for *M. tuberculosis* and a fresh infiltrate in the lateral segment of the right middle lobe (figure 22D). It was believed that this represented reactivation of a minute residual focus and that a thoracoplasty done at the time of resection might have prevented the reactivation.

Only one patient in this series had a combined phrenic nerve interruption and thoracoplasty (figure 20). This patient had extirpation of the middle and lower lobes on the right side, and the elevation of the hemidiaphragm fulfilled an important space-reducing function.

Pleural thickening and adhesions, pre-existing contraction of the hemithorax, and the presence of disease in the remaining parenchyma are additional factors that may modify expansion patterns.

The Bronchial Stump

Defects of the bronchial stump are usually attributed to faulty closure or infection (7). The latter may be intrinsic or extrinsic. Intrinsic infection usually is related to endobronchial tuberculosis at the site of transection. It is due less commonly to retention of secretion within the stump with subsequent mucosal damage, development of stitch abscesses, and eventual formation of bronchopleural fistula. These complications tend to occur in stumps with poor drainage, such as one in a dependent position (figures 14, 16, 20) (2) or one that is too long (figure 17) (4, 7). Extrinsic erosion by a tuberculous empyema secondary to contamination of the pleural space during surgery has been incriminated by some writers (7, 8, 9) as a significant mechanism of fistulation of the stump.

In performing a segmentectomy, the surgeon divides the segmental bronchus and removes the segment from the rest of the lung along the intersegmental planes. A subsegmentectomy is carried out in a similar manner. In either case the only stump is that of the segmental or subsegmental bronchus (figures 3, 4, 6, 7). In clamp excision or wedge resection, on the other hand, clamps are applied around the diseased area, which is then extirpated without regard to the segmental anatomy. Here, multiple bronchial branches are transected and multiple stumps thereby created (figures 2, 8, 9, 15). There is, no evidence, however, to indicate that the risk of stump complications might be correspondingly increased in the latter types of operation.

Bronchographic demonstration of pleural spaces containing pus and/or air depends upon the intracavitary pressure, the caliber of the communicating bronchopleural fistula, and the viscosity of the radiopaque medium. Visualization was achieved in 2 cases of the present series. In one, the spaces outlined assume semi-annular or oval shapes (figure 23). Such "pseudo-cavities" (9) may, on conventional roentgenograms, be mistaken for true parenchymal rarefactions.

Postoperative Bronchiectasis

Bronchiectasis in a previously normal segment or lobe has frequently appeared in subjects who have had excisional therapy for nontuberculous bronchiectasis (1, 3). The pathogenesis of this complication remains unsettled, but post-resection changes, i.e., pleural reaction, atelectasis, and impaired drainage due to bronchial distortion and displacement, are considered important factors (1, 3, 4). None of the patients in this study, however, developed new areas of bronchiectasis postoperatively, although many had "tuberculous bronchiectasis" before surgery. The present experience suggests that the surgical effects mentioned above play only a minor role in the postoperative recurrence of nontuberculous bronchiectasis, while some basic or "constitutional" cause is the real culprit (4).

Summary

The postoperative bronchograms of 52 tuberculous patients with various types of pulmonary resection are reviewed and the expansion patterns of each lung are illustrated.

Thoracoplasty is useful in preventing or correcting overexpansion and excessive distortion of the remaining parenchyma.

The pathogenesis of bronchopleural fistula and the visualization of abnormal pleural spaces are discussed briefly. Postoperative bronchiectasis in a previously uninvolved portion of lung was not observed in this series.

Sumario

Patrones Broncográficos Consecutivos a la Resección por Tuberculosis Pulmonar

Rapasados los broncogramas postoperatorios de 52 tuberculosos en quienes se ejecutaron varias

formas de resección pulmonar, se ilustran los patrones de expansión de cada pulmón.

La toracoplastia resulta útil para impedir o rectificar la hiperexpansión y la excesiva deformación del parénquima restante.

Se discuten sucintamente la patogenia de la fístula broncopleural y la visualización de los espacios pleurales anómalos. En esta serie, no se observó bronquiectasia postoperatoria en ninguna porción del pulmón, previamente inafectada.

Resume

Schémas bronchographiques après résection pour tuberculose pulmonaire

Des bronchogrammes post-opératoires chez 52 tuberculeux ayant subi diverses résections pulmonaires sont examinés et les schémas de dilatation de chaque poumon sont décrits.

La thoracoplastie est utile pour prévenir ou corriger une dilatation compensatrice exagérée ou une déformation excessive du parenchyme restant.

La pathogénie des fistules broncho-pleurales et la représentation d'espaces pleuraux anormaux sont discutés brièvement. Une bronchiectasie post-opératoire dans une région pulmonaire précédemment intacte n'a pas été observée chez ces malades.

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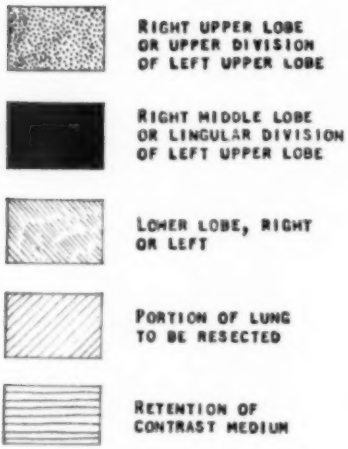

FIG. 1. Key to figures 2-20.

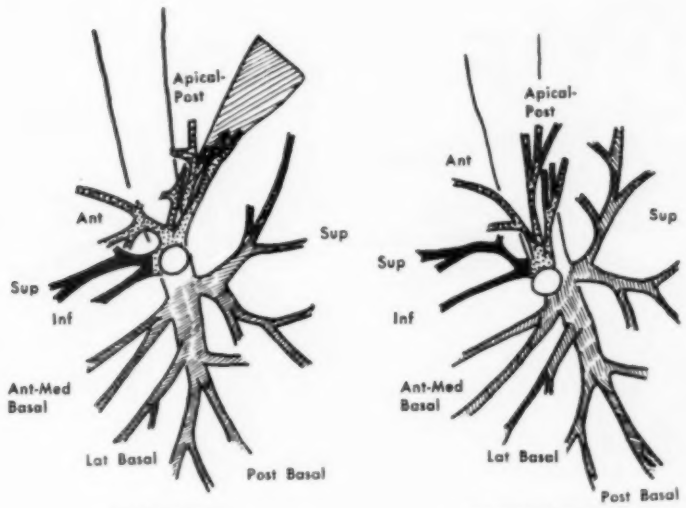

FIG. 2. Wedge resection (apical posterior segment, left upper lobe). Lateral views. Postoperative alteration of bronchial pattern is noticeable only when the wedge removed is large and deep, such as shown in this case. Here may be seen a slight upward shift of the anterior segment, lingula, and superior segment of lower lobe. The remainder of the apical posterior segment is displaced somewhat forward. Note that multiple bronchial branches have been transected, resulting in multiple bronchial stumps.

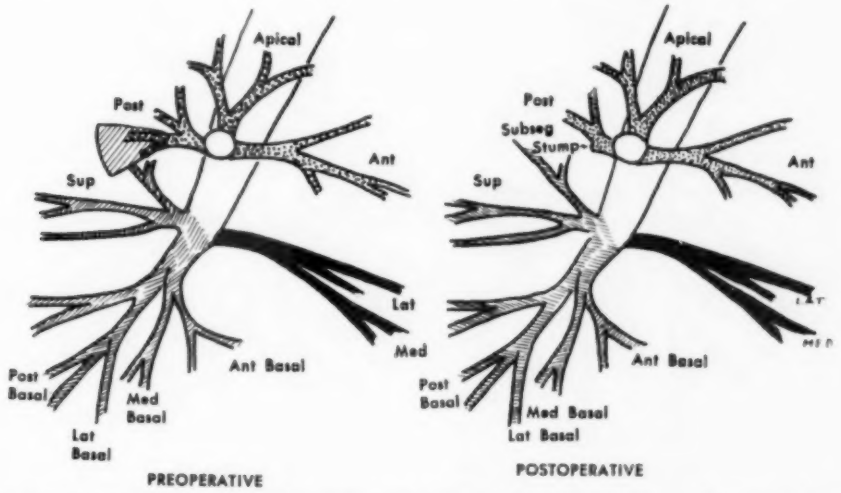

FIG. 3. Subsegmentectomy (posterior segment, right upper lobe). Lateral views. Removal of a subsegment causes little or no change in the bronchial pattern.

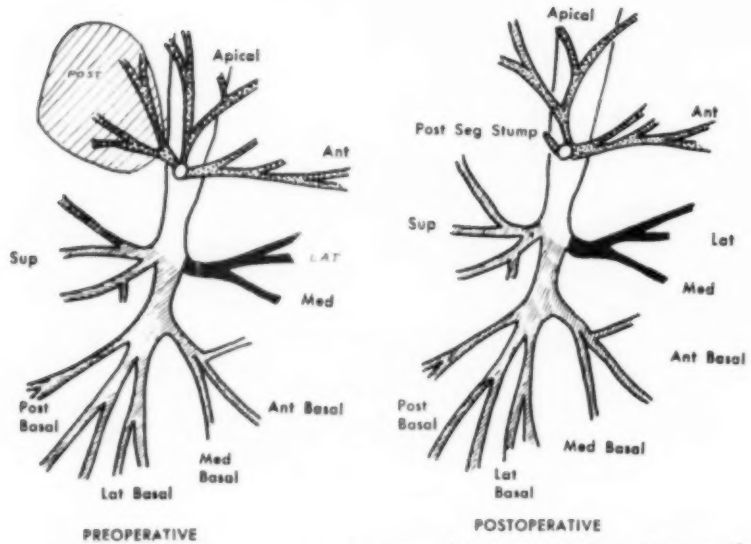

FIG. 4. Segmentectomy (posterior segment, right upper lobe). Lateral views. Postoperative distention and dorsad displacement of apical segment. Some rise of anterior segment. Slight upward expansion of superior segment and middle lobe.

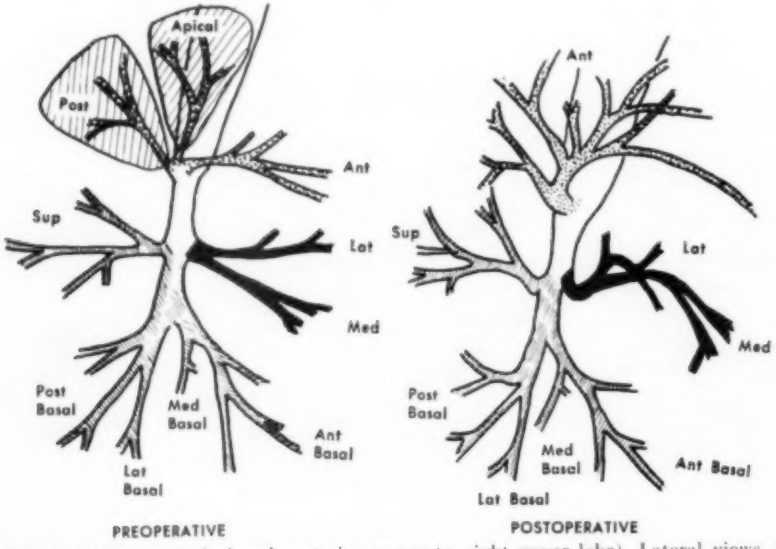

FIG. 5. Segmentectomy (apical and posterior segments, right upper lobe). Lateral views. Marked postoperative expansion and dorso-cephalad shift of anterior segment. Moderate upward displacement of superior segment and middle lobe. Note humping of middle lobe branches.

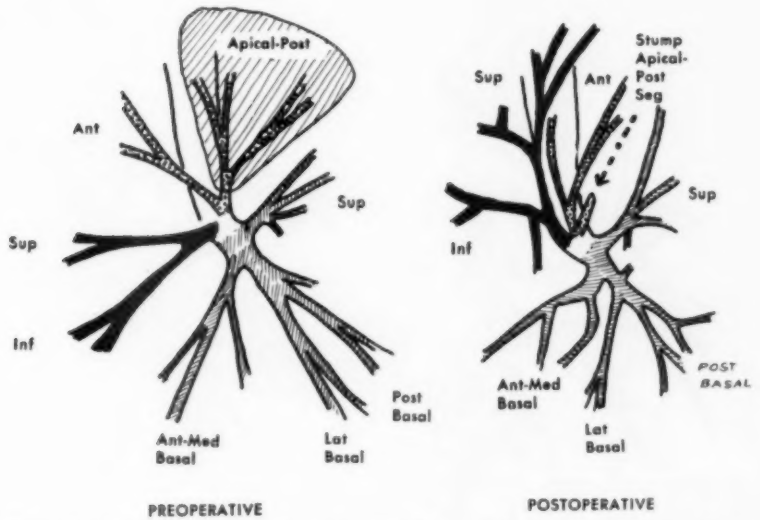

FIG. 6. Segmentectomy (apical posterior segment, left upper lobe). Lateral views. Postoperative backward shift and expansion of anterior segment. Similar but much more pronounced changes in lingula. Distention of lower lobe, especially the superior segment.

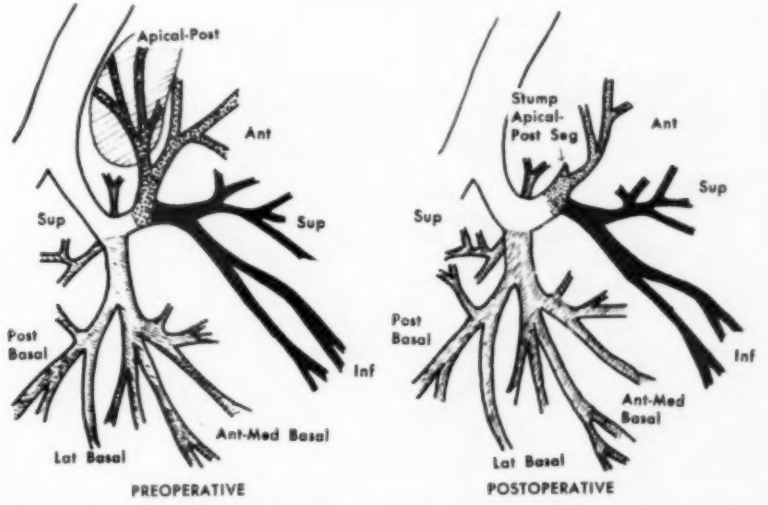

FIG. 7. Segmentectomy (apical posterior segment, left upper lobe) with thoracoplasty. Oblique views. Postoperative changes are minimized by the three-rib thoracoplasty. Slight dorso-cephalad shift of anterior segment is the only finding.

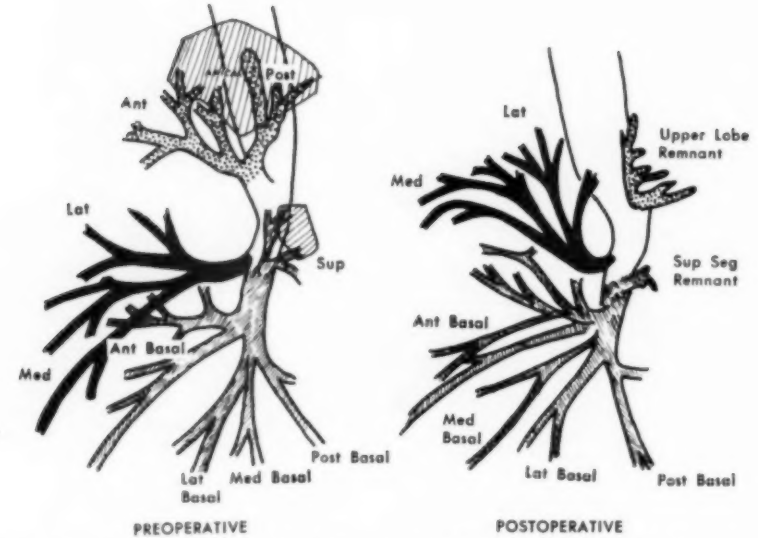

FIG. 8. Clamp excision (apical and posterior segments, right upper lobe, and superior segment, right lower lobe). Oblique views. The upper lobe remnant is displaced backward, middle lobe is shifted upward, and the basal segments are distended. Note multiple bronchial stumps.

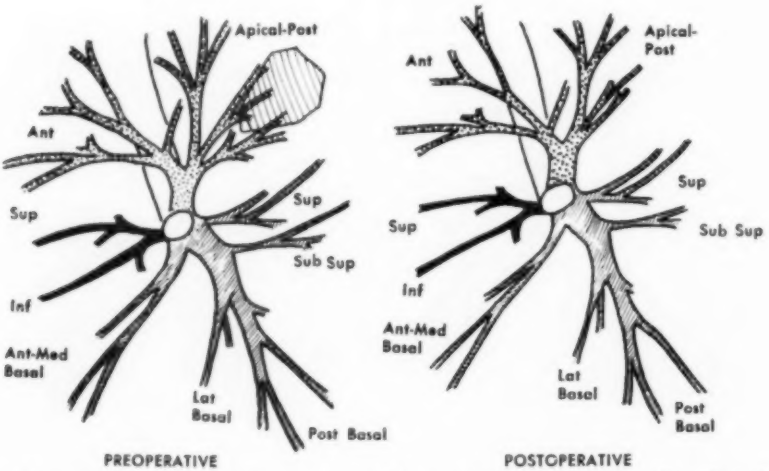

FIG. 9. Clamp excision (apical posterior segment, left upper lobe). Lateral views. Crowding of bronchi of remainder of apical posterior segment. Other segments are not affected. Changes are minimal because of the small amount of pulmonary tissue removed. Note again the multiple stumps resulting from transection of multiple bronchial branches.

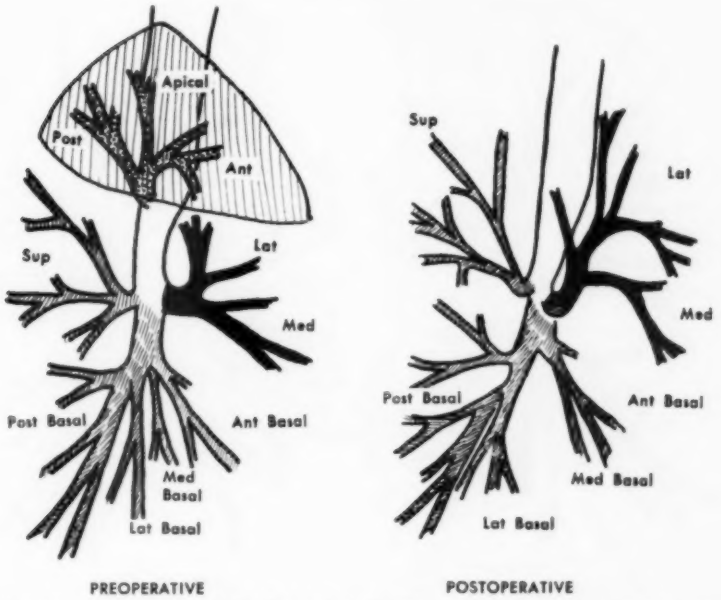

FIG. 10. Lobectomy (right upper lobe). Lateral views. The middle lobe and superior segment of the lower lobe become joint space-fillers by means of overexpansion and upward shift. Basal segments of lower lobe are distended. Note ventro-cephalad angulation of middle lobe bronchus with retention of contrast medium.

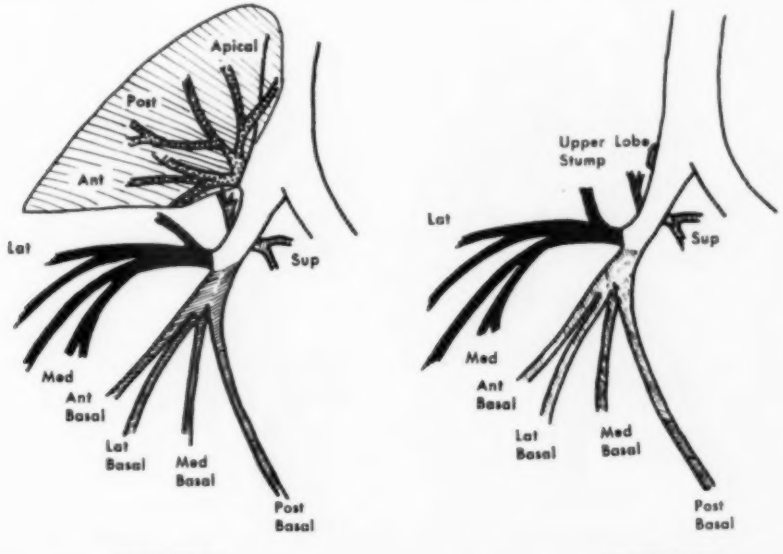

FIG. 11. Lobectomy (right upper lobe) with thoracoplasty. Frontal views. Postoperative changes are prevented by the four-rib thoracoplasty. On lateral bronchogram the upper lobe stump is seen pointing posteriorly.

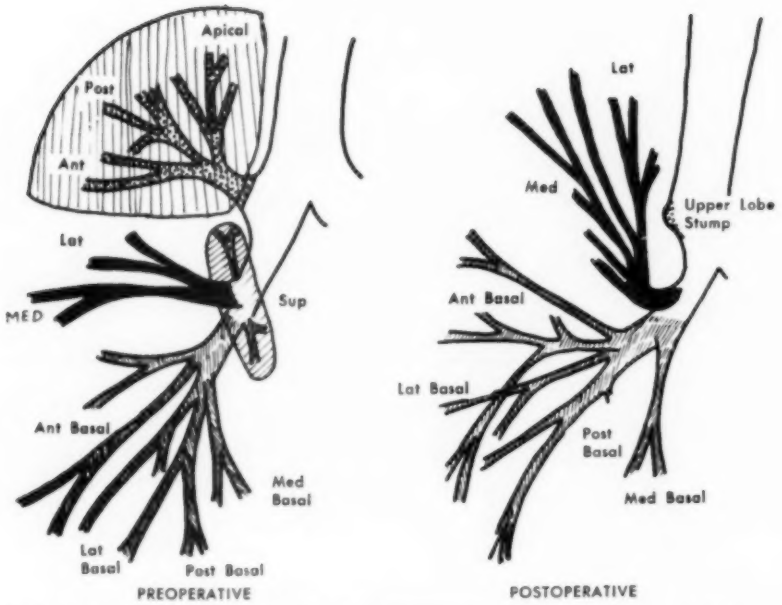

FIG. 12. Lobectomy (right upper lobe) and segmentectomy (superior segment, right lower lobe). Frontal views. Middle lobe has undergone marked distention and upward shift to become the major space-filler, with angulation of its bronchus and pooling of contrast medium at the curvature. Considerable expansion of all basal segments of lower lobe.

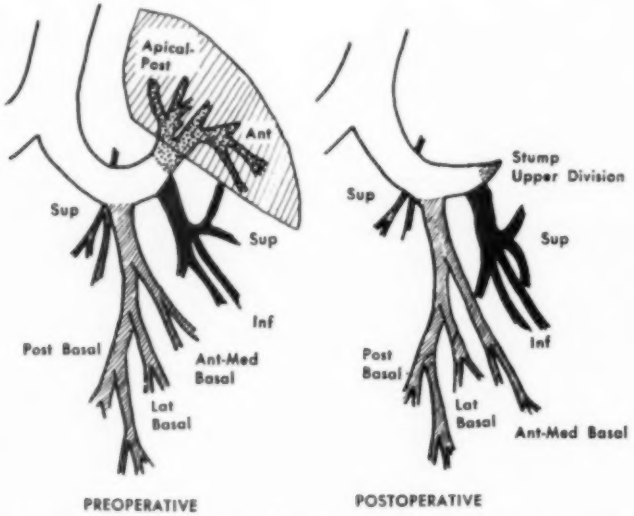

FIG. 13. Segmentectomy (apical posterior and anterior segments, left upper lobe) with thoracoplasty. Frontal views. This is the counterpart of a right upper lobectomy, and similar changes would have followed if the four-rib thoracoplasty had not been performed.

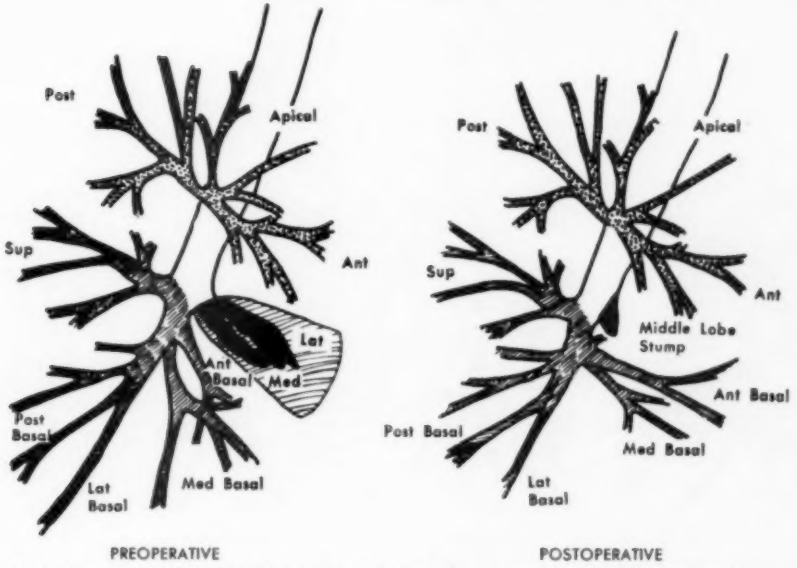

FIG. 14. Lobectomy (right middle lobe). Lateral views. Note preoperative shrinkage and bronchiectasis of middle lobe, with downward shift of anterior segment and some expansion of apical segment. These would have been part of the postoperative changes if the middle lobe had been normal in size. Cephalad displacement of anterior and medial basal segments after lobectomy.

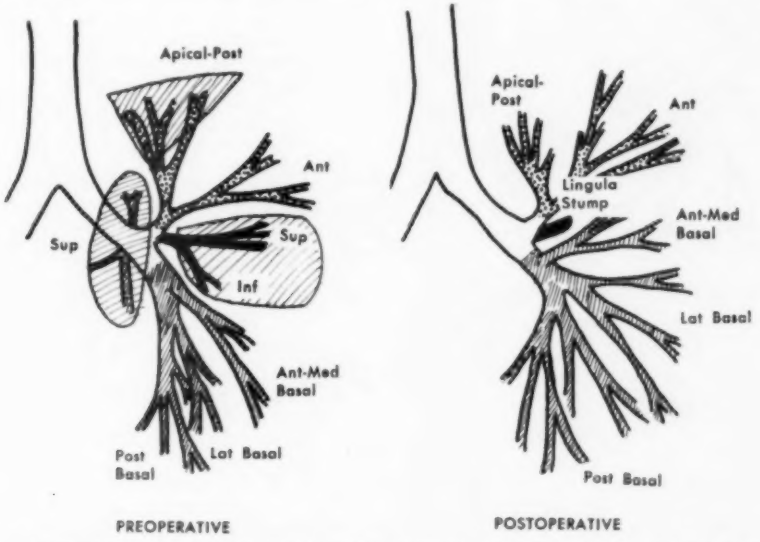

FIG. 15. Lingulectomy, segmentectomy (superior segment, left lower lobe), and wedge resection (apical posterior segment, left upper lobe). Frontal views. Remnant of apical posterior segment is shifted backward on lateral bronchogram. Enlargement and dorso-cephalad displacement of anterior segment. Stump of lingula is pulled upward and backward. Marked expansion of remainder of lower lobe with ascent of the anterior-medial basal segment.

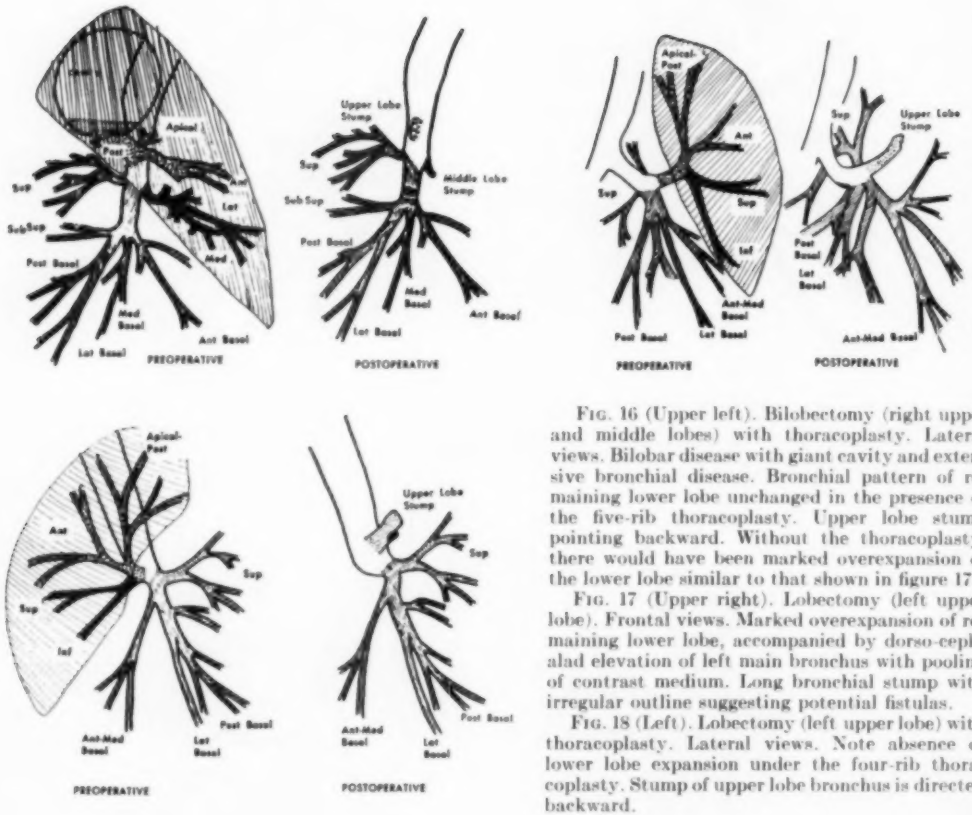

FIG. 16 (Upper left). Bilobectomy (right upper and middle lobes) with thoracoplasty. Lateral views. Bilobar disease with giant cavity and extensive bronchial disease. Bronchial pattern of remaining lower lobe unchanged in the presence of the five-rib thoracoplasty. Upper lobe stump pointing backward. Without the thoracoplasty, there would have been marked overexpansion of the lower lobe similar to that shown in figure 17.

FIG. 17 (Upper right). Lobectomy (left upper lobe). Frontal views. Marked overexpansion of remaining lower lobe, accompanied by dorso-cephalad elevation of left main bronchus with pooling of contrast medium. Long bronchial stump with irregular outline suggesting potential fistulas.

FIG. 18 (Left). Lobectomy (left upper lobe) with thoracoplasty. Lateral views. Note absence of lower lobe expansion under the four-rib thoracoplasty. Stump of upper lobe bronchus is directed backward.

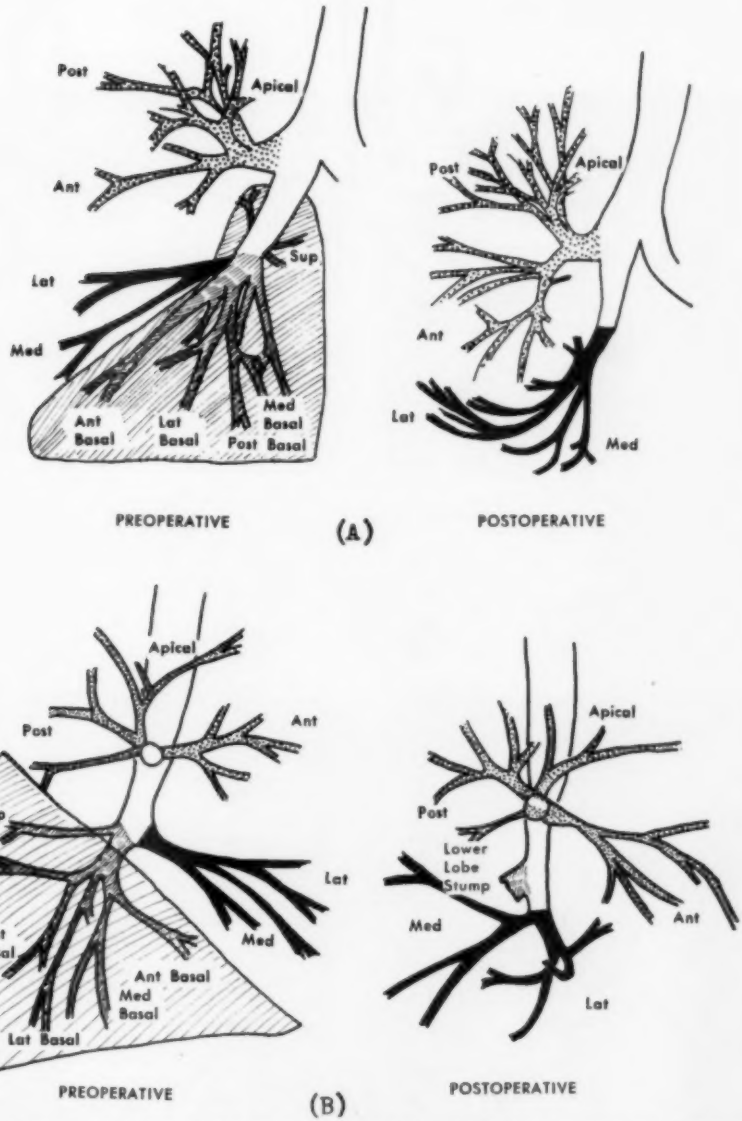

FIG. 19. Lobectomy (right lower lobe). Frontal (*A*) and lateral (*B*) views. Downward, backward, and inward sweep of the overexpanded middle lobe with bending ("kneeling") of its terminal branches (*A*). Considerable distention and ventro-caudad shift of upper lobe, especially the anterior segment.

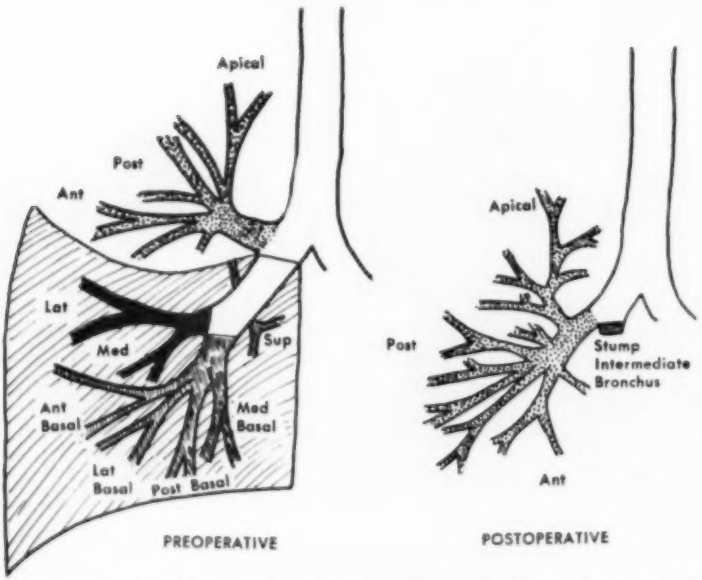

FIG. 20. Bilobectomy (right middle and lower lobes) with four-rib thoracoplasty and phrenic nerve interruption. Frontal views. Interval between surgery and bronchography: seven and a half years. Note expansion of all three segments of remaining upper lobe and retention of contrast medium (or sputum) in stump of intermediate bronchus. Mucosal swelling and redness in the stump were demonstrated on bronchoscopy. Sputum was positive for tubercle bacilli for the first time since surgery.

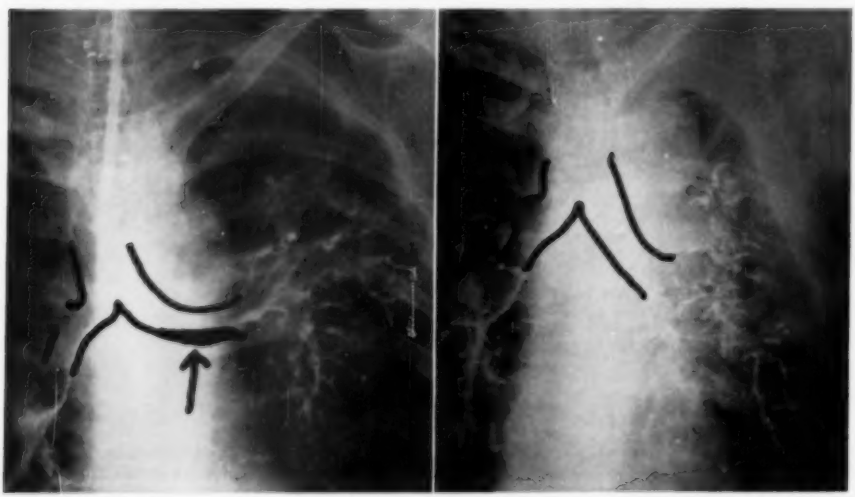

FIG. 21 (*A*) (Left). Preoperative bronchogram. Fibrotic contraction of left upper lobe causing (dorso-cephalad) elevation of left main bronchus with retention of contrast medium (arrow). (*B*) (Right). Postoperative bronchogram. Correction of the bronchial elevation by thoracoplasty concomitant with segmentectomy.

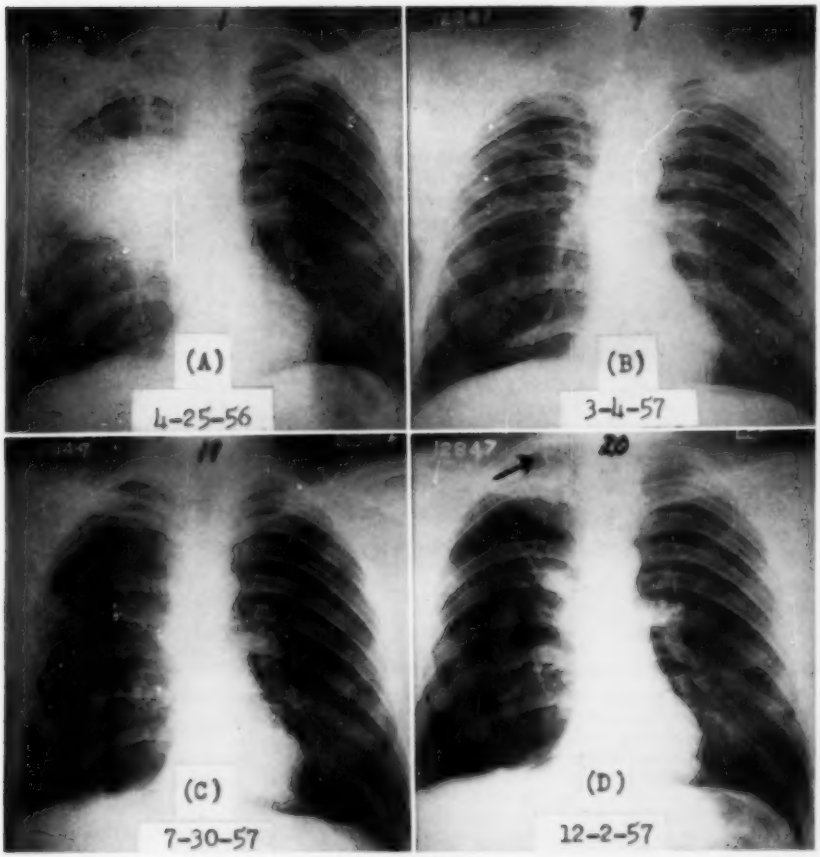

FIG. 22 (*A*). Admission roentgenogram of patient *M. F.* Extensive involvement of right lung.
(*B*). Preoperative roentgenogram. Remarkable resolution under antimicrobial therapy.
(*C*). Discharge roentgenogram. Four-plus months after resection of right upper lobe and superior segment of right lower lobe.
(*D*). Readmission roentgenogram. Fresh infiltrate (arrow) above right clavicle, where the lateral segment of middle lobe is now located (figure 12).

FIG. 23 (*A*) (Left). Frontal view. (*B*) (Right). Lateral view. Bronchographic demonstration of semi-annular pleural spaces (arrows) appearing after lingulectomy, segmentectomy of superior segment, and wedge resection of apical posterior segment of left lung (*see* figure 15). The communicating bronchopleural fistulas are not well visualized.

THE TREATMENT OF DEEP MYCOTIC INFECTIONS WITH AMPHOTERICIN B[1]

With Particular Emphasis on Drug Toxicity

HALLARD W. BEARD, JOEL H. RICHERT, AND RICHARD R. TAYLOR

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Introduction

The treatment of deep mycotic infections presents a difficult and challenging therapeutic problem. Many antifungal agents have been evaluated and most have been discarded because of insufficient antifungal action, inordinate toxicity, or difficulty of administration. Penicillin (in the treatment of nocardiosis and actinomycosis) and the diamine compounds (in the treatment of North American blastomycosis) are notable exceptions. Amphotericin B is a newer agent which is presently undergoing clinical investigation, and it has been proved to have antifungal activity both *in vitro* and *in vivo*.

Reports of therapeutic success with amphotericin B have dealt principally with the treatment of cryptococcosis (1–14), coccidioidomycosis (1–3, 15–18), histoplasmosis (1, 2, 4, 14, 19), and blastomycosis (1, 14, 20). In these studies, amphotericin B was administered orally, intravenously, or in combination. In general, the most rapid and lasting therapeutic responses were obtained with intravenous therapy. Other deep mycotic infections which may be benefited by treatment with amphotericin B are aspergillosis, geotrichosis, maduromycosis, systemic moniliasis, and sporotrichosis.

The present study was undertaken to evaluate the therapeutic efficacy and the physiologic toxicity of amphotericin B in the treatment of deep mycotic infections. Although the work of other investigators indicates the value of amphotericin B in the treatment of deep mycotic infections, the evaluation of this antifungal agent is far from complete because of the small number of suitable cases available for study.

Materials and Methods

The cases of deep mycotic infection selected for treatment with amphotericin B[2] were in the following categories:

Disseminated deep mycotic infections caused by any of the pathogenic fungi.

Localized chronic progressive deep mycotic infections, caused by any of the pathogenic fungi which had shown by their clinical course that a spontaneous cure or cure by ordinary methods was not to be expected.

Deep mycotic infections in which the natural course might be shortened by treatment with amphotericin B.

The amphotericin B administered was a water-soluble salt for intravenous injection.

Prior to therapy, all patients were evaluated by careful history and physical examination. The following baseline laboratory determinations were obtained: complete blood count; urinalysis; complete tests of liver function; total serum proteins, including albumin and globulin fractions; serum protein electrophoresis; blood urea nitrogen; serologic tests with histoplasmin, coccidioidin, and blastomycin; roentgenograms of diseased areas; cultures of fungi from diseased areas; and pulmonary function tests when indicated. When it became apparent that the administration of amphotericin B produced a definite elevation of blood urea nitrogen, the urine concentration test, phenolsulfonphthalein dye excretion test, and urea and creatinine clearance tests were added to the group of basic laboratory determinations.

Amphotericin B was administered daily in six-hour intravenous infusions in a dose of approximately 1 mg. per kg. of body weight for periods of four to six weeks. A dose of approximately 1 mg. per kg. of body weight per day was considered the optimal dose. The calculated daily dose of amphotericin B was given in a liter of 5 per cent dextrose in water.

All laboratory determinations were repeated at two-week intervals throughout hospitalization. The blood pressure, temperature, respiratory rate, and pulse rate of each patient were measured four times daily. The patient's weight was measured weekly. Any change in the patient's symptoms or physical findings was noted daily.

[1] From the Department of Medicine and the U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons Army Hospital, Denver 30, Colorado.

[2] Amphotericin B was kindly supplied by The Squibb Institute for Medical Research, New Brunswick, New Jersey.

When clinical and laboratory improvement occurred in the course of the patient's illness, it was construed as being produced by amphotericin B therapy. Worsening or no change in the patient's condition was viewed as a therapeutic failure. Acute toxic reactions to amphotericin B were noted at the times of infusion. The symptoms, physical signs, and abnormalities of laboratory determinations occurring during therapy, but not present prior to therapy or related to the fungal infection, were considered as toxic manifestations secondary to amphotericin B therapy. The patients included in this study were followed as long as practicable after the completion of therapy.

Observations

Response to Therapy

Seven patients with chronic pulmonary coccidioidomycosis, one patient with disseminated blastomycosis, and one patient with chronic pulmonary histoplasmosis were treated with amphotericin B at Fitzsimons Army Hospital between March, 1957, and April, 1959 (table 1). The duration of disease prior to therapy ranged from six months to eight years, with an average duration of three years. In each case, the disease was identified prior to therapy by culture or by examination of tissue sections.

The response to therapy was considered good in 4 cases of pulmonary coccidioidomycosis and excellent in the case of disseminated blastomycosis (*Cases 1*, *2*, *3*, *5*, and *9*, table 1). At the completion of therapy, these patients were symptomatically and physically improved. Cultural negativity and roentgenographic improvement were attained in each case. The periods of follow-up observation of these patients range from five to eighteen months. The disease in 4 of these patients has not relapsed; one patient (*Case 5*, table 1) had a cultural relapse ten months after the completion of therapy.

The patient with pulmonary histoplasmosis had no response to therapy (*Case 4*, table 1). In addition to extensive parenchymal disease, he had a fibrous mediastinitis. Following therapy, there was no detectable improvement in his illness. The response to therapy was considered poor in one case of cavitary pulmonary coccidioidomycosis (*Case 8*, table 1). Although there was a decrease in the size of the cavity and the sputum became negative for *Coccidioides immitis* there was a cultural relapse two months following the completion of therapy.

Two patients with pulmonary coccidioidomycosis were given four weeks of therapy to prevent local recurrence following surgical resection of their primary lesions (*Cases 6* and *7*, table 1). Although neither patient developed this complication, it was not possible to determine the value of amphotericin B in the management of their cases.

Toxicity of Amphotericin B

The amphotericin B administered to the patients in this study did not adversely affect the following laboratory determinations: leukocyte count, differential cell count, hemoglobin level, hematocrit, sedimentation rate, total and direct serum bilirubin, serum cholesterol and esters, serum alkaline phosphatase, cephalin flocculation, thymol turbidity, bromsulphalein dye retention, Kunkle test, prothrombin activity, total serum proteins, or electrophoretic patterns of serum proteins.

Routine urinalysis, on occasion, showed abnormalities. Three patients had intermittent albuminuria of a trace to one-plus in amount; two other patients had isolated albuminuria. None of these patients had albuminuria before or after treatment. During therapy, the specific gravity of routine urine specimens ranged from 1.001 to 1.030. After three weeks of therapy, all patients had hyposthenuria with maximal urinary specific gravity of 1.015. Frequently, the urine sediment contained leukocytes, but this finding was not consistent and bore no relationship to the period of treatment. No patient had microscopic hematuria. Five patients intermittently passed hyaline casts in their urine during the course of treatment, but not before or thereafter.

Amphotericin B produced a definite elevation of blood urea nitrogen during each course of therapy (figure 1). Normal concentrations were present in all 9 patients prior to therapy, and this elevation returned to normal in each case after the completion of therapy.

The urea clearance, phenolsulfonphthalein dye excretion, and urine concentration tests were obtained before, during, and after the treatment of 8 patients (figures 2, 3, and 4). All determinations were normal prior to therapy. During the course of therapy and concomitant with the elevation of blood urea nitrogen, there was a significant decrease in the ability of all patients to clear urea, excrete phenolsulfonphthalein dye, and concentrate urine. These abnormalities cleared after the completion of therapy.

Creatinine clearance was measured before,

TABLE 1

NINE PATIENTS TREATED WITH AMPHOTERICIN B

Case	Age, Race, and Sex	Disease	Therapy	Response
1	30 W M	Coccidioidomycosis—5 years. Recurrent lung cavity after two pulmonary resections, empyema, bronchopleural fistula, sputum positive for *C. immitis*	2.1 gm. 6 weeks	Good 1. Symptoms improved; cultural relapse after 3 months
			2.1 gm. 6 weeks	2. Pneumonectomy covered with amphotericin B; well after 18 months
2	24 W F	Coccidioidomycosis—3 years. Recurrent lung cavity following surgery, sputum positive	2.1 gm. 6 weeks	Good Cavity closed, sputum negative; well after 18 months
3	17 N M	Blastomycosis—6 months. Disseminated, lung, skin, bone, joint, genitourinary; cultures and tissue sections positive	2.1 gm. 6 weeks	Excellent All lesions healed; well after 18 months
4	27 W M	Histoplasmosis—5 years. Extensive disease of lung, hemorrhagic endobronchitis, fibrous mediastinitis, tissue sections positive	2.1 gm. 6 weeks	None No detectable response
5	29 O M	Coccidioidomycosis—2 years. Bilateral apical cavitary disease, sputum positive	2.1 gm. 6 weeks	Good Symptomatic and roentgenographic improvement; cultural relapse after 10 months
6	21 W M	Coccidioidomycosis — 6 months. Right upper lobe cavity removed by segmental resection, tissue sections positive	1.4 gm. 4 weeks	Unknown Postoperative course uncomplicated; well after 10 months
7	39 N M	Coccidioidomycosis—8 years. Left upper lobe cavity removed by lobectomy, tissue sections positive, postoperative fever and pleural effusion	1.4 gm. 4 weeks	Unknown Fever gone in 1 week, effusion gone in 6 weeks, no recurrence; well after 10 months
8	29 W M	Coccidioidomycosis—18 months. Large cavity, left upper lobe, sputum positive	2.1 gm. 6 weeks	Poor Cavity reduced in size, cultural relapse after 2 months; asymptomatic after 10 months
9	35 W F	Coccidioidomycosis—2 years. Recurrent right middle lobe and right lower lobe disease after right upper lobectomy, empyema, bronchopleural fistula, sputum positive	2.1 gm. 6 weeks	Good Symptomatic improvement; cultures negative after 5 months

during, and after the treatment of 6 patients (figure 5). Four of these patients showed a temporary reduction in their ability to clear creatinine during treatment.

All 9 patients had persistent anorexia and frequent nausea throughout therapy. Nausea was most pronounced during the last half of the infusion or immediately thereafter. Seven of the patients occasionally vomited. These symptoms were particularly marked during the first few weeks of therapy and ceased shortly after therapy was completed. The degree of nausea was directly proportional to the rate of administration of the intravenous infusion. The daily administration

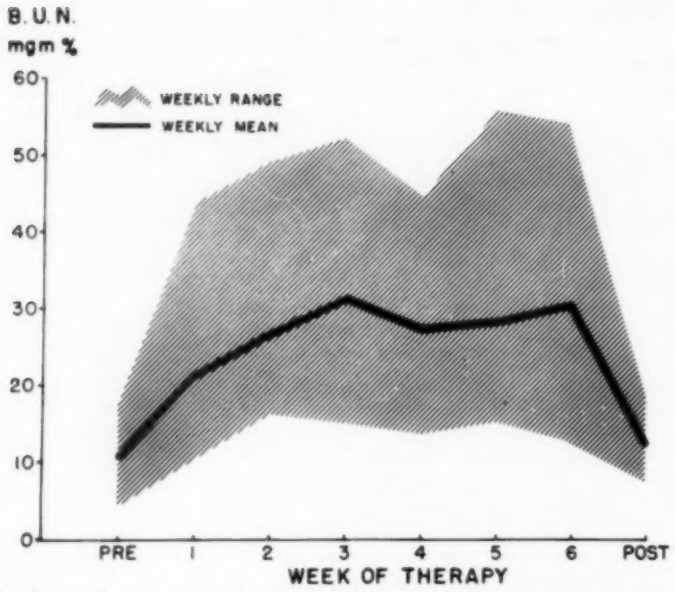

FIG. 1. Blood urea nitrogen before, during, and after therapy with amphotericin B (9 cases).

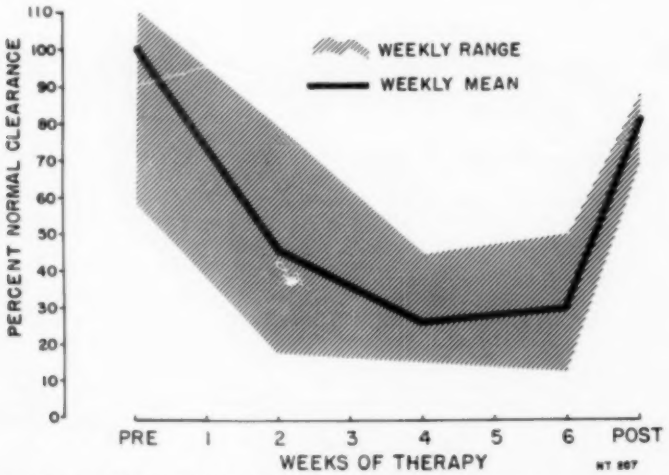

FIG. 2. Urea nitrogen clearance before, during, and after therapy with amphotericin B (8 cases).

of dimenhydrinate (Dramamine®) prior to the intravenous infusion did not diminish the degree of anorexia or nausea.

Eight of the patients experienced acute toxic reactions during the intravenous administration of amphotericin B. The patient who received two courses of therapy experienced acute toxic reactions only during his first course of therapy. These reactions occurred forty-five to two hundred and forty minutes after the infusion was started and lasted between two and four hours. Their onset was usually abrupt, with

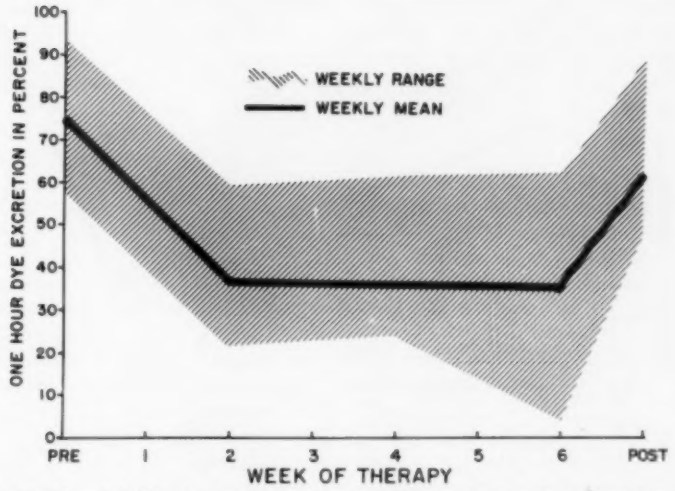

FIG. 3. Phenolsulfonphthalein dye excretion before, during, and after therapy with amphotericin B (8 cases).

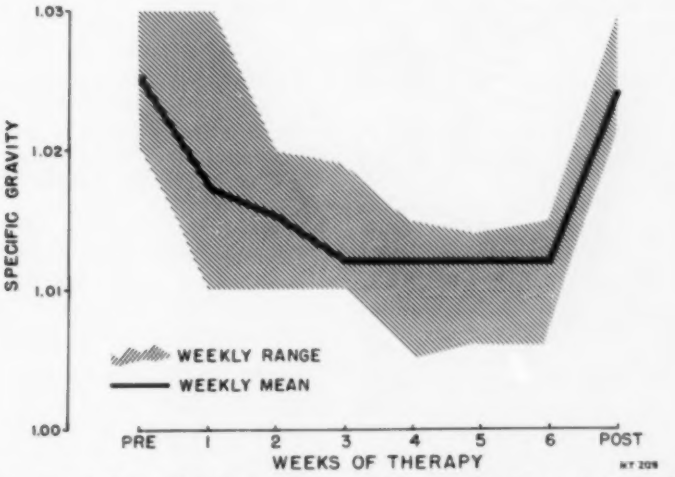

FIG. 4. Urine concentration test before, during, and after therapy with amphotericin B (8 cases).

nausea and frequent vomiting. Usually, the patient was apprehensive and complained of a chill or a chilly sensation. One patient had clonic muscle contractions which required the intravenous injection of sodium amobarbital (Amytal®) for control. The systolic and diastolic blood pressures became elevated from 20 to 40 mm. of mercury, but returned to normal soon after the reaction. The temperature rose to 101° or 102°F. and remained elevated from one to two hours. A tachycardia of 110 to 140 was frequently present. The respiratory rate usually showed only slight acceleration. Not all reactions were of equal severity. A mild reaction consisted of acute nausea, with or without vomiting, and a chilly sensation; but no changes in pulse rate, blood pressure, respiratory rate, or temperature. The severe reactions occurred during the first

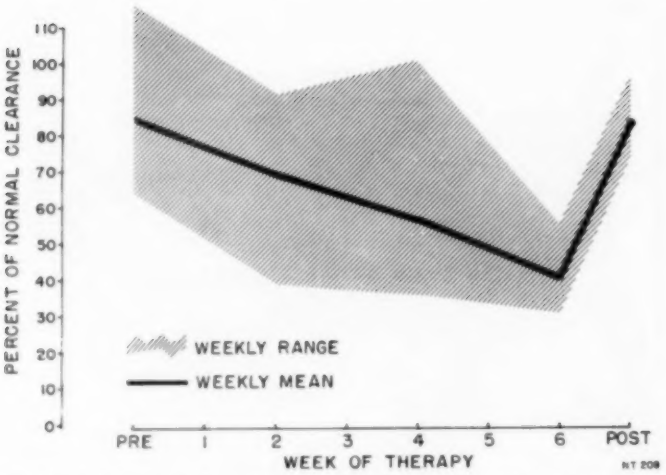

FIG. 5. Creatinine clearance before, during, and after therapy with amphotericin B (6 cases).

week of administration, and subsequent reactions were usually of decreasing severity. The intravenous infusion was discontinued when a severe reaction occurred. The rate of administration was reduced during mild reactions. Fifty milligrams of diphenhydramine (Benadryl®) and 10 gr. of acetylsalicylic acid were given during reactions and also as a prophylactic measure; but their effect could not be evaluated.

Amphotericin B is irritating to tissues and produces local pain and inflammation at the site of administration. All 9 patients experienced mild to moderate pain at the site of intravenous infusion. One patient required analgesics and the application of ice packs for relief of pain. Superficial erythema of the skin and tenderness of the vein persisted for twenty-four hours after administration. This was most marked during the first few weeks of therapy and gradually subsided. One patient consistently developed a palpable venous thrombus at the site of infusion. He did not receive anticoagulants. The superficial venous structure of all 9 patients became progressively poorer and more difficult to infuse. The course of therapy of the patient with disseminated blastomycosis could be completed only by giving the infusion through a polyethylene catheter which was inserted into the femoral vein. It is not known whether or not the tissue-irritating factor of amphotericin B is contained in its antifungal fraction. It may be contained in an impurity which will be eliminated with improved refinement.

DISCUSSION

The evaluation of the response of any patient with deep mycotic infection to an antifungal agent is difficult. Clinical improvement coinciding with therapy is not necessarily an indication of therapeutic response because these diseases, by nature, are subject to spontaneous periods of remission. Clinical improvement followed by the absence of disease recurrence is of final importance. It is the impression of the writers that 5 of the patients treated with amphotericin B had definite remission of their disease.

Prior to therapy, 5 patients consistently produced sputum from which *Coccidioides immitis* was cultured. After two to three weeks of therapy with amphotericin B, *Coccidioides immitis* could no longer be cultured. This indicates that at least three weeks of therapy are required for the disappearance of the fungi from the sputum. This may be important in the preoperative management of patients with localized pulmonary coccidioidomycosis when sputum conversion is desired prior to pulmonary surgery.

Localized chronic pulmonary coccidioidomycosis may be removed and cured surgically. Frequently, the nature of such a lesion is unknown prior to surgery and is recognized only

after pathologic examination. The surgical removal of these lesions is complicated by recurrence of disease in the same area, particularly during the immediate postoperative period. This complication seems most imminent when endobronchial disease is present and the sputum culture is consistently positive for *Coccidioides immitis*. In addition, the lesion of localized chronic pulmonary coccidioidomycosis is not solitary but is surrounded by many small granulomas disseminated throughout the lung parenchyma. As the major lesion is resected, the endobronchial disease and many of the small satellite granulomas are transected. This may stimulate recurrence of disease in the same area. Under these circumstances, a period of therapy with amphotericin B prior to surgical resection may be justified.

Amphotericin B has a definite suppressive effect on normal renal physiology. It produces a decrease in both glomerular and tubular function. Apparently, this is temporary and clears rapidly following the completion of therapy. At no time did the blood urea nitrogen of any of the patients rise appreciably above 50 mg. per 100 ml., even though therapy was continued daily at a constant dosage. At no time did the renal function deteriorate to the point of frank renal insufficiency of serious nature. It is the opinion of the writers that the elevation of blood urea nitrogen and the impairment of renal function induced by therapy with amphotericin B are not, in themselves, a contraindication to the continuation of therapy.

The indications for treatment with amphotericin B must be definite before a course of therapy is undertaken, since the difficulties of administration and the distressing side effects of the drug are many. All of the patients treated in this study had persistent anorexia and frequent nausea throughout therapy. On occasion, this led to poor dietary intake and increased debility. The venous structure of each patient became progressively poorer and more difficult to infuse. The acute toxic reactions to amphotericin B contributed greatly to the patient's fear and apprehension. All of the patients objected to the length of the course of therapy. This was arbitrarily set at a period of four to six weeks. Although improvement may occur in a shorter period of time, the disease is probably not controlled by periods of therapy shorter than four to six weeks. The optimal period of therapy is unknown and must be individualized on the merits of a particular case. It may be that, in certain cases, the period of therapy should be much longer than six weeks and, in this respect, comparable with the length of antituberculous therapy.

Serum concentrations of amphotericin B were measured during the course of therapy of 6 patients. In each case, effective antifungal serum concentrations persisted between periods of infusion. Amphotericin B could not be demonstrated in the serum or the urine after intramuscular or oral administration. At present, intravenous administration is the route of choice.

Summary

Seven cases of chronic pulmonary coccidioidomycosis, one case of disseminated blastomycosis, and one case of chronic pulmonary histoplasmosis treated with a new antimicrobial, amphotericin B, are presented. Definite clinical improvement was attained in 4 of the cases of chronic pulmonary coccidioidomycosis and in the one case of disseminated blastomycosis. Four of these patients have not had recurrence of disease. Whether or not a cure has been attained will not be known until after sufficient follow-up study.

Albuminuria and cylindruria were frequently noted during the course of therapy. Of particular interest is the significant elevation of blood urea nitrogen which occurred during each course of therapy. More elaborate urinary function tests showed an inability to concentrate urine, excrete phenolsulfonphthalein dye, and clear blood urea and creatinine during therapy. All renal abnormalities cleared after the completion of therapy. Acute toxic reactions frequently occurred during the period of intravenous infusion of amphotericin B. Not all reactions were of equal severity. The severe reactions occurred during the first weeks of administration, and subsequent reactions were of decreasing severity. There were no residual clinical stigmata following the completion of therapy.

Sumario

El Tratamiento de las Micosis Profundas con Anfotericina B, con Atención Particular a la Fármacotoxicidad

Preséntanse 7 casos de coccidioidomicosis pulmonar crónica, 1 caso de blastomicosis diseminada

y 1 caso de histoplasmosis pulmonar crónica, tratados con un nuevo antimicrobiano, la anfotericina B. Se obtuvo mejoría clínica bien definida en 4 de los casos de coccidioidomicosis pulmonar crónica y en el caso de blastomicosis diseminada. Cuatro de estos enfermos no han tenido recurrencias de las enfermedad. No se sabrá si se ha alcanzado o no la curación sino después de un adecuado estudio de observación subsiguiente.

Notáronse frecuentemente albuminuria y cilindruria durante el transcurso de la terapéutica. Revistió interés en particular la notable elevación del nitrógeno de urea en la sangre, que se observó durante cada serie de terapéutica. Pruebas más delicadas de la función urinaria revelaron incapacidad para concentrar la orina, excretar el colorante fenolsulfonftaleína y eliminar la urea y la creatinina sanguíneas durante la terapéutica. Todas las anomalías renales desaparecieron después de terminar la terapéutica.

Hubo frecuentemente reacciones tóxicas agudas durante el período de inyección intravenosa de la anfotericina B. No fueron todas ellas de gravedad igual. Las graves tuvieron lugar durante las primeras semanas de la administración de la droga, y las subsiguientes alcanzaron menor gravedad. No hubo estigmas clínicos residuales después de terminada la terapéutica.

Resume

Le traitement des affections fungiques graves par l'amphotéricine B, et analyse de la toxicité du produit

Les auteurs présentent sept cas de coccidioïdomycose, un cas de blastomycose généralisée, et un cas d'histoplasmose pulmonaire chronique, traités par un nouvel antifungique, l'amphotéricine B. Une amélioration clinique nette a été obtenue dans 4 des cas de coccidioïdomycose pulmonaire chronique et dans un cas de blastomycose généralisée. Quatre de ces malades n'ont pas eu de récidive, mais seule l'observation des suites pendant un temps suffisant permettra de dire si la guérison obtenue est définitive ou non.

Au cours du traitement on note souvent de l'albuminurie et de la cylindrurie. Il faut noter en particulier l'augmentation significative de l'azote de l'urée sanguine survenant au cours de chaque traitement. Des tests plus minutieux de la fonction urinaire montrent un défaut de concentration de l'urine, de l'excrétion de la phénolsulfonephtaléine et l'élimination de l'urée sanguine et de la créatinine pendant le traitement. Toutes les anomalies rénales disparurent après cessation du traitement.

Des réactions toxiques aiguës, d'intensité variable, se sont produites souvent pendant l'injection intraveineuse d'amphotéricine B. Les réactions graves se produisirent pendant les premières semaines du traitement et diminuèrent par la suite. Aucune séquelle clinique ne fut constatée après la fin du traitement.

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A MORPHOLOGIC STUDY OF MYCOBACTERIA INFECTED WITH D32 PHAGE[1, 2]

KONOSUKE FUKAI AND MARGRET I. SELLERS

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INTRODUCTION

In earlier electron microscopic studies of mycobacteriophage (1), micrographs were obtained which suggested that a study of the intracellular development of phage might reveal information on the morphogenesis of virus particles. Phage D32 was selected because it is the largest of the four mycobacteriophages studied (head diameter, 80 mμ and tail length, 220 mμ) and has the longest latent period. The latent period is approximately one hundred minutes, the rise period lasts for twenty minutes, and lysis occurs one hundred and twenty minutes after adsorption of phage to bacteria (2).

MATERIALS AND METHODS

A saprophytic *Mycobacterium* sp. ATCC 9033 was employed as host. The cells were grown at 37°C. with aeration in heart infusion broth containing 0.002 M calcium chloride for four to five hours. When the culture had reached a density of 5×10^7 cells, 10 phage particles per bacterium were added. Adsorption was allowed to proceed for ten minutes, at the end of which time 17 per cent of the phage had been adsorbed; the remaining free phage was neutralized with antiserum. At intervals, aliquots of the infected culture were withdrawn and processed for electron microscopic examination. In similar experiments, *Mycobacterium tuberculosis* H37Rv was employed as the host bacterium except that the culture, in enriched heart infusion broth, was shaken on a wrist type of shaker for approximately forty-eight hours before phage was added and sampling begun.

In the preparation of D32 phage particles for sectioning, high-titered lysates were purified by alternating cycles of high-speed centrifugation (1). The final sedimented phage "button" was fixed in osmium tetroxide and processed for examination in the electron microscope as described below.

Preparation of specimens: At specific intervals, 9-ml. aliquots of infected culture were removed and fixed immediately by adding 1 ml. of 2.0 per cent osmium tetroxide buffered at pH 7.4 according to the method of Birch-Andersen and associates (3). The specimens were dehydrated in graded dilutions of ethyl alcohol (thirty minutes in each dilution) and embedded in epoxy resin by a modification of the method of Glauret and co-workers (4) as follows:

Materials

Resin—Hysol 2030[3]
Catalyzer—Hardener A[3]
Plasticizer—Dibutyl phthalate

Infiltration

(*1*) Eight hours at room temperature in a mixture of 10 parts resin, 5 parts ethanol, 2.5 parts plasticizer
(*2*) Repeat step 1.

Embedding

Cure at 60°C. for twenty-four to thirty hours in a mixture of 10 parts resin, 2.5 parts plasticizer, 1 part catalyzer.

Sections were cut on a Porter-Blum microtome, floated onto the surface of a 20 per cent acetone-80 per cent water mixture, and picked up on collodion-coated grids. The specimens were examined in a Hitachi Hu-9C electron microscope.

OBSERVATIONS

Cultures of mycobacteria show marked inhomogeneity of the cell population. This can be demonstrated even in the light microscope and, to a greater extent, by electron microscopic examination. In order to ascertain and compare the numbers of abnormal or ghost cells present in the two bacterial populations, air-dried specimens of both normal and phage-infected bacteria were examined in the electron microscope. Cells from an uninfected culture are shown in figure 1, and uranium-shadowed cells from the same culture may be seen in figure 3. Figures 2 and 4 are comparable micrographs of phage-infected bacteria. Considerable numbers of micrographs were taken, and numbers of intact cells and cells which showed morphologic alterations were counted; these results are recorded in table 1.

[1] From the Nina Anderton Laboratory for Electron Microscopy, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, California.

[2] This study was supported, in part, by grants from the Los Angeles County Tuberculosis and Health Association, the California Tuberculosis and Health Association, and the San Diego County Tuberculosis and Health Association.

[3] Hysol 2030 and Hardener A are products of the Houghton Laboratory, Inc.

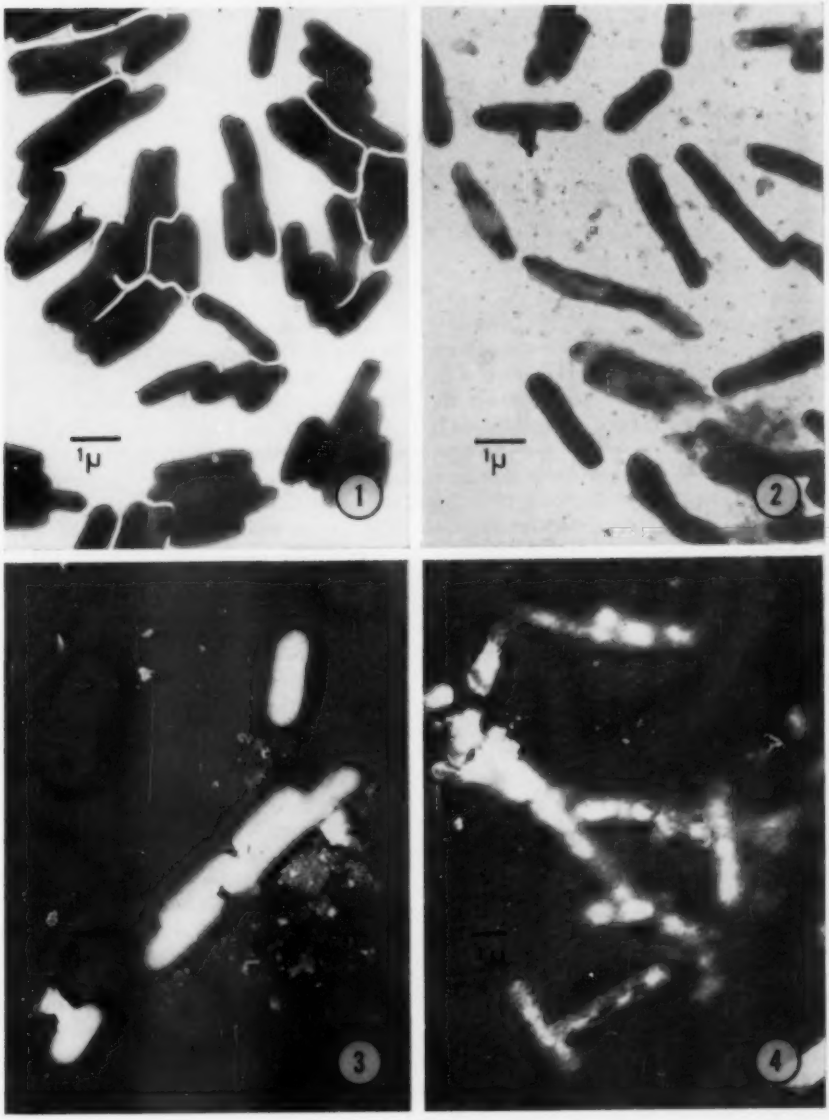


PLATE I

FIG. 1. Cells from a culture of host bacteria, *Mycobacterium* sp. ATCC 9033.

FIG. 3. U-shadowed cells from the same culture.

FIGS. 2 and 4. Comparable micrographs of D32 phage-infected bacteria. Although morphologically altered or ghost cells may be found in a normal cell population, they appear approximately eight times more frequently in a phage-infected culture.

TABLE 1

MORPHOLOGICALLY ALTERED CELLS IN NORMAL AND IN PHAGE-INFECTED MYCOBACTERIA

Population	Number of Microscope Grids Examined	Number of Cells Observed	Number of Altered Cells	
			Total	Per Cent
Normal	10	901	35	3.9
Infected	21	2,127	633	29.7

Approximately 4 per cent of cells in the normal culture showed morphologic changes, compared with 30 per cent in the phage-infected culture. It was concluded that the numbers of morphologically altered cells were sufficiently greater in the infected bacteria to make the contemplated study feasible.

In attempts to detect morphologic changes in the internal structure of infected cells during phage development, samples were taken prior to the addition of phage and from the beginning of the latent period, just after infection, to the middle of the rise period. Several blocks for sectioning were prepared from each sample, and approximately 50 electron micrographs were made per block. Regardless of time after phage infection when samples were taken, the resultant micrographs did not show one single kind of cell but cells which had several shapes and structures. In general, the morphologic appearance of the cells could be classified into four groups, *A*, *B*, *C*, and *D*. The characteristics of each group are as follows:

Type A: The cell has a clearly defined cell wall and cytoplasmic membrane. It is filled with homogeneous protoplasm with a cavity in the center. In the center of the cavity an electron-dense, "nuclear" structure is seen (figures 5, 7, 8 and 9).

Type B: The cell has a well-defined cell wall and cytoplasmic membrane also. The inside of the cell is filled with homogeneous protoplasm, but no special structures exist (figures 6, 8, and 10).

Type C: The cell shows no morphologic change in the cell wall; but particles ranging in size close to D32 phage heads, after allowing for shrinkage during fixation, are embedded in the homogeneous protoplasm. In this cell, the nuclear structure is not detected (figures 8 and 9).

Type D: The cell may show a disrupted cell wall and appear to be devoid of the usual intracellular contents, or it may appear to be vacant. In some cases, several granules or electron-dense bodies of the approximate size of D32 phage heads were found just inside the cell wall (figures 8, 10, and 11).

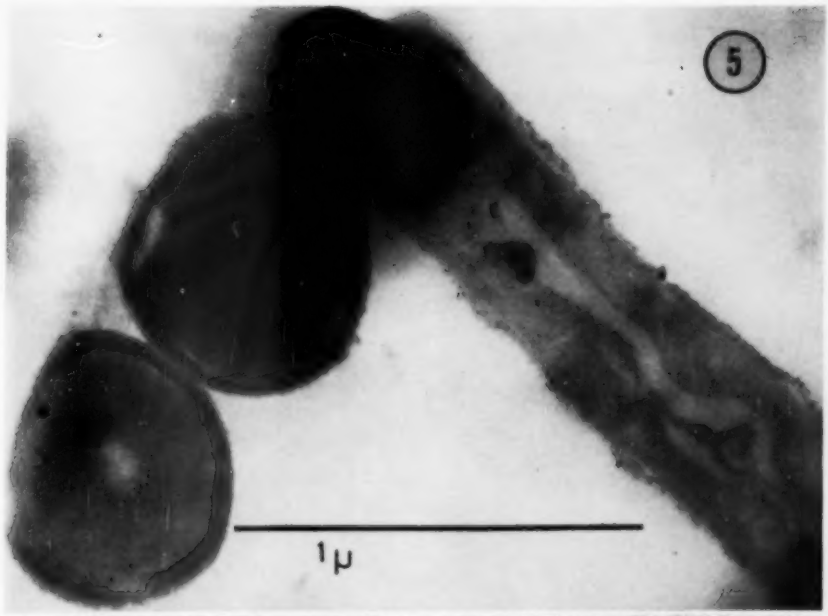
Among the different cell types, *Type C* appeared to show the resultant products of phage synthesis. Also, *Type D* may indicate bacteriolysis resulting from phage reproduction.

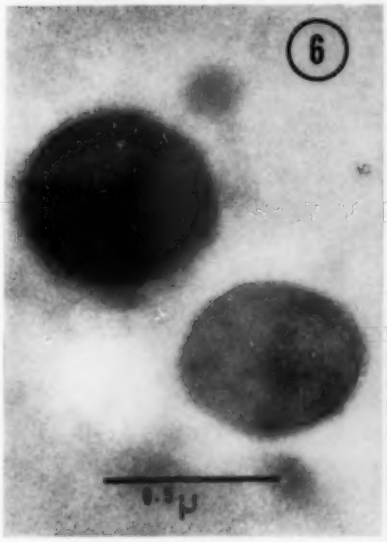
In order to obtain more objective information concerning the significance of the cell types with respect to phage reproduction, the cells in the electron micrographs were classified into the groups described, and the ratio of the appearance of each group at each sampling time was tabulated. The results of these analyses are recorded in table 2. The numbers of *Type A* cells begin to decrease at about ninety minutes after infection. The appearance of *Type B* cells is independent of sampling time. *Type C* cells were not found in samples taken soon after infection or in the control samples, and their numbers increased with time after infection. The appearance of *Type D* cells was constant with time.

The results of the analysis expressed in percentages show these tendencies more clearly than actual numbers. It was postulated that *Type A* cells were normal, intact cells and that *Type C* cells were phage-infected. *Type B* cells are probably sections through the end areas of bacteria. Because bacterial lysis results from phage multiplication, it would be expected that the ratio of *Type D* cells would increase with sampling time; this was not the case, and the ratio of these cells remained fairly constant. When the processes employed in preparing the cells for sectioning are taken into account, however, it is suggested that lysed cells and cell debris, because of their low density, would tend to be lost during the repeated centrifugations, especially after successive infiltrations of the samples with heavy resin mixtures.

Using virulent mycobacteria (H37Rv) as the host, similar cell types were observed (figure 11). However, data from this experiment were insufficient for quantitative analysis.

Of considerable interest were the photomicrographs of sections made from purified preparations of D32 phage and illustrated in figure 12. Sections through the head portion of phage showed an outer coat composed of homogeneous protoplasm contained within a limiting membrane, both on the inside and outside. The inner core appeared to be vacant; this could be a result of the sectioning process when, because of the compressing action of the microtome knife, the





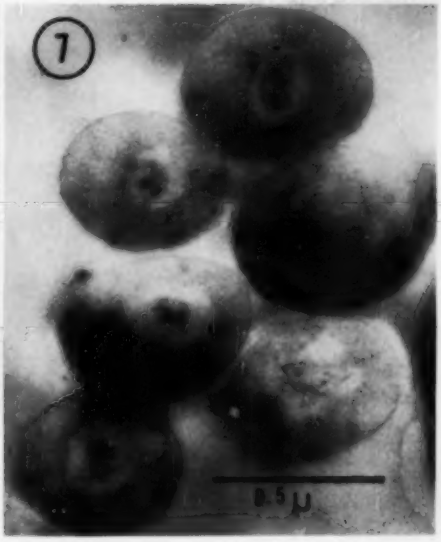


PLATE II

FIG. 5. Sagittal and cross sections of normal host bacteria. In contrast to all other micrographs shown in this study, this section was from a 48-hour culture grown on a solid medium. It is included here to show the appearance of the nuclear structure in the center of the bacterium in the longitudinal aspect; and to show the osmophilic property of thickened, electron-dense cell walls of two bacteria seen in cross-section. The latter phenomenon was not observed in cells from a culture in an exponential growth phase.

FIG. 6. *Type B* cells showing a well-defined cell wall and cytoplasmic membrane. The inside of the cell is filled with homogeneous protoplasm.

FIG. 7. *Type A* cells: These have clearly defined cell walls and cytoplasmic membranes and are composed of homogeneous protoplasm in the center of which is a vacuole or cavity. Within the cavity, electron-dense nuclear structures are seen. It is probable that what appears to be a vacuole is in reality an artifact produced by fixation of the bacteria.

Sections shown in figures 5, 6, and 7 were made from noninfected cultures.

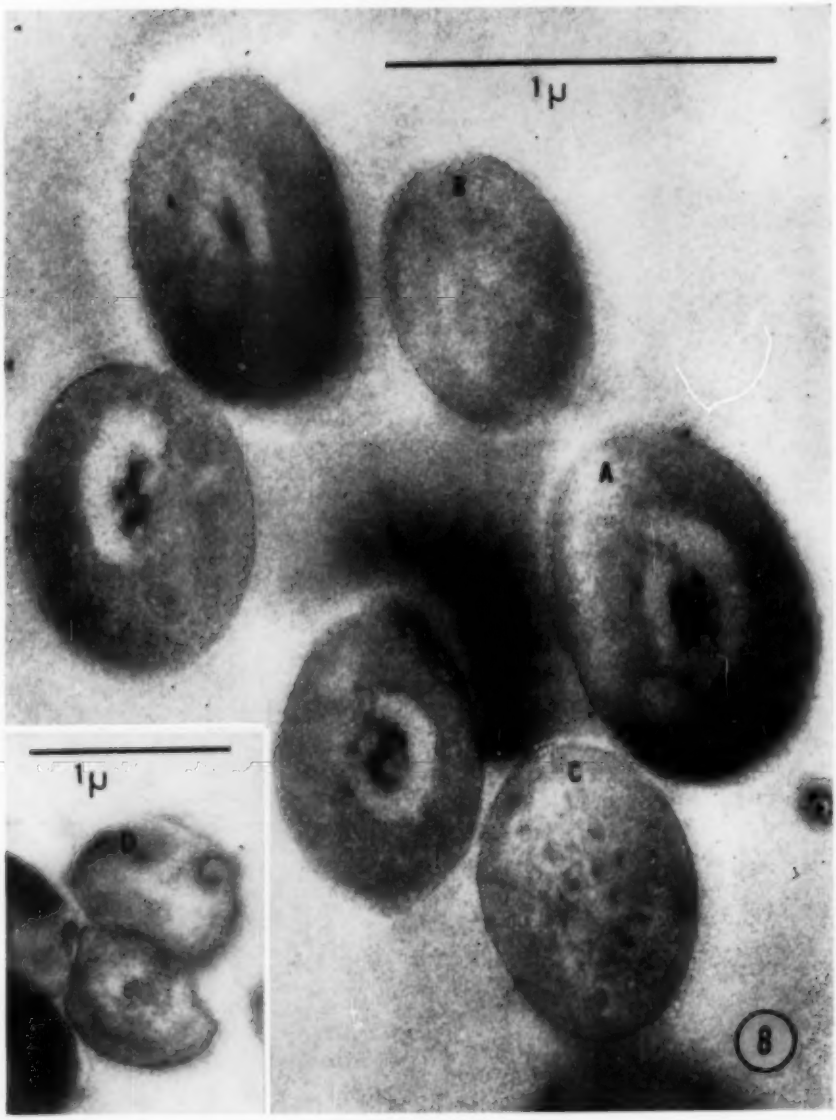


PLATE III

FIG. 8. All of the cell types described, *A*, *B*, *C*, and *D*, are seen in this figure. (*Type A* cells are described for figure 7; *Type B*, for figure 6.) *Type C* cells show no morphologic changes in the cell wall, but particles ranging in size close to D32 phage heads are embedded in the homogeneous protoplasm; shrinkage resulting from fixation undoubtedly produces the clear space around the phage particles. In this cell type, the nuclear structure is not detected. The *Type D* cell shows a ruptured cell wall and is devoid of much of its intracellular cytoplasm.

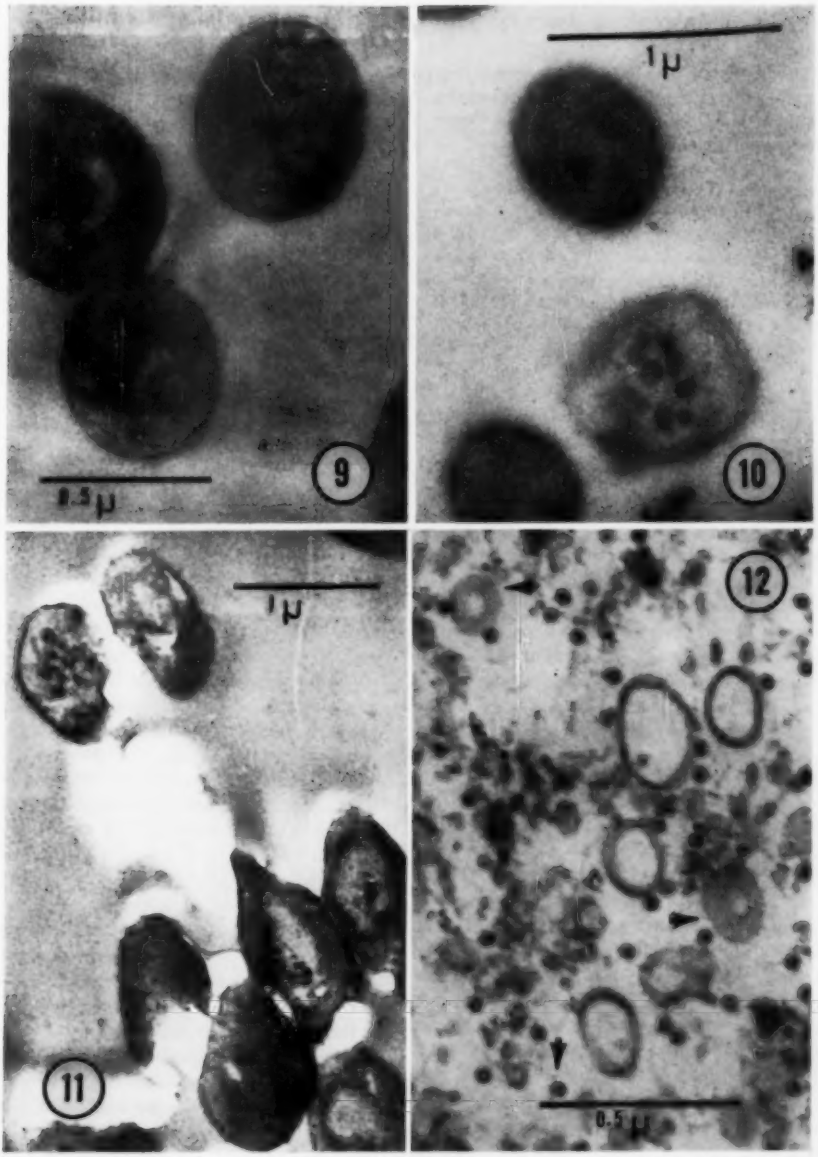


PLATE IV

FIG. 9. Two *Type A* cells and one *Type C* cell.

FIG. 10. A cell showing morphologic changes in the cell wall. It is devoid of the usual intracellular material and appears to contain phage particles. Two characteristic *Type B* cells are also present.

FIG. 11. Section of bacteria from a 48-hour culture of *M. tuberculosis* (H37Rv) infected with D32 phage. The cell wall is osmophilic and the cell is filled with electron-dense phage particles.

FIG. 12. Section of D32 mycobacteriophage. Arrows point to two phage heads in cross section and to a cross section of a phage tail (numerous other tail cross sections are scattered throughout the micrograph). In both structures an electron-opaque central area is surrounded by a well-defined outer coat. The opaque central area in the head probably represents space occupied by the nucleic acid core but which has become detached and lost during the sectioning process. The same process could also account for the empty central core of the tail.

TABLE 2
TYPES OF CELLS FOUND IN A MYCOBACTERIUM SP. CULTURE AT DIFFERENT INTERVALS AFTER D32 PHAGE INFECTION

Time*	Number of Blocks Examined	Number of Cells Observed	Cell Types Present							
			A		B		C		D	
			Total	Per Cent	Total	Per Cent	Total	Per Cent	Total	Per Cent
0	5	1,680	1,300	77.4	372	22.1	0	0	8	0.5
10	5	1,886	1,395	74.0	484	25.7	0	0	7	0.4
20	3	1,113	849	76.3	247	22.2	2	0.2	15	1.3
45	5	1,622	1,286	79.2	313	19.3	0	0	23	1.4
70	6	1,548	1,161	75.1	362	23.4	7	0.5	18	1.2
90	7	2,643	1,896	71.7	486	18.4	212	8.0	49	1.9
95	6	2,406	1,704	70.8	450	18.7	209	8.7	43	1.8
120	10	2,776	1,917	69.1	554	20.0	262	9.4	43	1.5

* In minutes after phage infection.

central core became detached and lost. If it is assumed that the morphology of D32 mycobacteriophage is similar to that of T2 coliphage (5), then the combination of nucleic acid core with outer protein coat appears to be easily disrupted. Cross sections of phage tails also showed a vacant core. Whether this indicates that the tail core was composed of a continuation of the nucleic acid of the head portion of the phage particle is being investigated further.

DISCUSSION

In an electron microscopic study of *Escherichia coli* infected with T_4 coliphage, Maaløe and associates (6) found that the nuclear apparatus of the cell was immediately affected in a way suggesting loosening of, and fusion throughout, the cell of the material normally tightly wound in the center of distinct vacuoles. In mycobacteria infected with mycobacteriophage, distinct particles which are of the approximate diameter of D32 heads are found; the nuclear structure is also no longer distinct. At no time were these particles found in cells with intact nuclear structures. It was hoped that the time at which phage protein first appears in infected cells could be established by serologic techniques. However, premature lysis experiments with mycobacteria were not successful. Lysozyme plus versene treatment (7) had only a slight effect on the cells even when the action was allowed to proceed overnight. Sonic vibration was of little value, as were alternate freezing and thawing in distilled water. However, seventy minutes after infection the host cells could be ruptured by treatment with osmic acid or by sonic vibration, suggesting that some change occurs in the cell wall which facilitates disruption. At this time a few mature phage particles begin to appear.

Observation of many sections showed that cross sections of cells predominated and that sagittal sections were rare. Because all sections were cut perpendicularly to the long axis of the block, this indicates that the bacterial cells were arranged in a parallel orientation along the long axis of the block as a consequence of the previous centrifugation.

In sections of mycobacteria from a four- to five-hour broth culture in the log phase of growth, a distinct outer envelope or cell wall 80 to 100 Å in width was observed. The cell wall appeared to consist primarily of polysaccharide as judged by its electron-scattering property, which is much less than would be expected if it were composed of proteins or lipids. In osmium tetroxide-impregnated specimens, the latter two components appear much darker than that observed in the cell wall. In addition, an electron-dense cytoplasmic membrane was easily seen. Similar observations have been made by Kellenberger and Ryter in ultrathin sections of *E. coli* (8). On the other hand, samples of mycobacteria taken from a forty-eight-hour culture on a solid medium (*see* figure 5) revealed some cells with cell walls of increased thickness (100 to 200 Å), a highly osmophilic property, and consequent increased density. It is possible that the so-called lipid nature of the cell walls of mycobacteria is associated with, or is dependent upon, aging.

SUMMARY

Significant numbers of a D32 phage-infected mycobacterial population show no changes for

fifty minutes after infection, as revealed by ultrathin sections in the electron microscope. At approximately seventy minutes after infection, electron-dense bodies of about the same diameter as D32 phage heads become visible in the infected cells. These particles are not found in uninfected cells and their numbers increase with time in the infected population. An intact nuclear structure was not found in cells which contained virus-like particles. Sections of mature phage indicate that the combination of nucleic acid with a protein coat is not very firm.

These observations of phage-infected mycobacteria are essentially similar to those found in *E. coli*-coliphage studies by other investigators.

Sumario

Estudio Morfológico de las Micobacterias Infectadas con el Bacteriófago D32

Proporciones importantes de una población micobacteriana infectada por el bacteriófago D32 no muestran alteraciones por 50 minutos después de la infección, según revelan cortes ultradelgados en el microscopio electrónico. Unos 70 minutos después de la infección, se vuelven visibles en las células infectadas cuerpos de espesor electrónico y aproximadamente del mismo diámetro que el fago D32. Estas partículas no se encuentran en las células indemnes y aumentan en número con el tiempo en la población infectada. No se descubrió un tejido nuclear intacto en las células que contenían partículas viroideas. Cortes de bacteriófagos maduros indican que no es muy firme la combinación del ácido nucleico con una capa de proteína.

Estas observaciones de micobacterias infectadas por fagos son en el fondo semejantes a las contenidas en los estudios del *E. coli-coliphagum* por otros investigadores.

Resume

Etude morphologique des mycobactéries infectées par D32-phage

Un nombre important de mycobactéries infectées par D32-phage ne présente aucune modification pendant 50 minutes après l'infection, ainsi que le montrent des coupes ultra-minces au microscope électronique. Au bout de 70 minutes, des corpuscules, environ du même diamètre que les têtes du D32-phage, deviennent visibles dans les cellules infectées. Ces particules ne se trouvent pas dans les cellules qui ne sont pas infectées et leur nombre augmente avec le temps dans les mycobactéries infectées. On n'a pas trouvé de structure nucléaire intacte dans les cellules contenant des particules ressemblant à des virus. Des coupes de phage adulte montrent que la combinaison de l'acide nucléique et du revêtement protéique n'est pas très stable.

Ces observations de mycobactéries infectées par des phages ressemblent beaucoup à celles faites par d'autres auteurs sur le *E. coli-coliphage*.

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A COMPARATIVE STUDY OF THE CAUSATIVE AGENT OF A MYCOBACTERIAL DISEASE OF SALMONOID FISHES[1]

I. A Comparison of the Staining Characteristics of the Fish Disease with Human Tuberculosis in Sections Stained by the Fite-Faraco and Ziehl-Neelsen Methods

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INTRODUCTION

Investigation of the etiologic agent of a mycobacterial disease of fish, usually referred to in the literature as "fish tuberculosis," has been undertaken by this laboratory. The initial phase of study consisted of indicating the presence of the organism in salmonoid fishes and describing the gross pathology of the disease (1). The present report is the result of work prompted by the observation that tissue sections made of organs that were strongly positive for acid-fast bacilli, when smeared and stained, were rarely positive after routine histologic techniques and staining by the Ziehl-Neelsen method. Further impetus was given the investigation when it was observed that the staining characteristics of the organism differed in juvenile and adult chinook salmon (*Oncorhynchus tshawytscha*).

An additional problem was presented by the host tissue in its retention of the dye. One of the outstanding differences in the staining of organisms in fish and human tissue (the control system) was the affinity of the fish tissue for the carbolfuchsin stain. Even long periods of time in strong decolorizing agents failed to remove it entirely, particularly in the younger fish. Some of the methods stained the tissue to such an extent that the dye could not be removed sufficiently to differentiate some of the normal host-cell characteristics.

The phenomenon of acid-fastness has been intensively studied, especially in relation to the human tubercle bacillus. Koch (2) stained the bacteria in an alkaline solution of methylene blue. In the same year Ehrlich (3) employed a solution of aniline oil-basic fuchsin or aniline oil-methyl violet for staining, and postulated that the property of acid-fastness is due to the presence of an alkali-permeable capsule surrounding the bacillus. Ziehl (4) disproved this theory when he stained the organism with an alcoholic methyl violet-aqueous carbolic acid solution. Neelsen (5) used carbolfuchsin in place of the methyl violet-carbol solution and decolorized with sulfuric acid instead of nitric acid to evolve the Ziehl-Neelsen technique.

Anderson (6) related the property of acid-fastness to a specific compound which was called mycolic acid. Knaysi (7) believed that the cytoplasmic membrane was the site of the staining property. Yegian and his co-workers studied the problem over a long period of time and concluded that the property was: (*1*) due to the composition of the dye (8); (*2*) due to the techniques employed for preparing and staining the material (9); and (*3*) that most of the dye responsible for the deep red color is free in solution within the cytoplasm (10) and not bound to any chemical such as mycolic acid. Most of these workers agree that acid-fastness is a property of the intact cell. From all of the investigations, the knowledge of the mechanism of the staining characteristic is still in a state of flux. Lamanna (11) summed up the problem nicely with the opening statement of his paper when he stated "A satisfactory explanation of the nature of the Ziehl-Neelsen stain (acid-fastness) remains to be formulated." For a complete and engaging discussion of the chemistry involved, see Drea and Andrejew's *The Metabolism of the Tubercle Bacillus* (12).

A review of the literature provided only one reference in which a staining technique for acid-fast bacilli in fish tissue was elaborated (13). When the present writers used Johnstone's methods on known positive tissue, negative results were obtained. Faraco, in 1938, published a technique which involved the concept of restoring acid-fastness to the leprosy bacillus after it had been rendered nonacid fast in the imbedding and deparaffinizing process. Heating the sections in vegetable oil returned the acid-fast

[1] From the Western Fish Disease Laboratory, U. S. Fish and Wildlife Service, Seattle, Washington.

properties to the organism. Wade (14) employed a similar method which he believed involved the concept of protecting the acid-fast properties of leprosy bacilli. He originally used essential oils throughout the process, beramount or origanum oil for deparaffinizing, and anise oil for clearing the sections after staining; but abandoned this method in favor of one devised by Fite, Chambre, and Turner (15) which was developed to improve on Faraco's refatting method (16). Wade (14) maintained, however, that the modifications of Fite and associates were, nevertheless, ones of protection rather than of refatting.

The present report is submitted not only as a presentation of results obtained from experimental work, but also to bring to the attention of those persons concerned with the detection of acid-fast bacilli in tissue that well-established, standard methods are not always best. Modern chemotherapy may render the organism poorly or nonacid fast, or the presence of atypical organisms may be unobserved due to the lack of proper staining techniques; in each instance the presence of disease goes undetected.

Materials and Methods

Tissue sections were obtained from the following sources and preserved in the indicated fixatives:

Juvenile chinook salmon (Oncorhynchus tshawytscha)

A. Spontaneous infection (hatchery-reared fish)
- 1. livers } 10 per cent buffered formalin
- 2. whole fish }

B. Experimental infections (laboratory-reared fish)
- 1. livers } Bouin's fixative
- 2. whole fish }

Adult chinook salmon

A. Spontaneous infection (fish returning from high seas to spawn)
- 1. livers—10 per cent buffered formalin

Human tissue

A. Tuberculosis
- 1. lung—10 per cent buffered formalin

Material from the spontaneously infected, juvenile, chinook salmon had been preserved in 10 per cent formalin for twenty-four months prior to its use. The rest of the material had been obtained one to four months prior to its use in this experimental work. Whole fish (two to three inches in length) from the spontaneous hatchery infection were decalcified in 5 per cent nitric acid for six hours so that sagittal sections could be made from them.

Fish obtained from the experimentally infected group had been fed cultures of acid-fast bacilli which had been isolated from salmonoid fish by members of this laboratory. All of the specimens used showed acid-fast bacilli in at least 10 per cent of the tissue.

The standard Ziehl-Neelsen and the Fite-Faraco techniques were the basis for the experimental work. The attempted modifications of these methods fall into four main groups: (*1*) pretreatment of the prepared sections, (*2*) varied concentrations of the decolorizing agents, (*3*) different counterstaining methods, and (*4*) combinations of the first three.

When a tissue was to be pretreated, the standard technique was employed up to the point at which the slide was normally placed in the stain. At that time the slides were subjected to pretreatment with glycerol, absolute alcohol, and/or 6 per cent sulfuric acid for ten minutes for each reagent. The sections were stained for twenty minutes in a cold solution of stain composed of 10 ml. of a 10 per cent alcoholic, basic-fuchsin solution in 100 ml. of 5 per cent aqueous phenol and were then decolorized until a faint pink color was obtained in the tissue, using either 1 per cent hydrochloric acid in 70 per cent alcohol or a 3 per cent aqueous solution of sulfuric acid. To complete the procedure, tissues were: not counterstained; counterstained with a 0.1 per cent methylene blue; or counterstained with a 1 per cent aqueous solution of Bismark brown. The protocols employed for the treatment of prepared slides are listed in table 1.

Results

The micropathology of the disease will be described in detail in a later paper. The following is a brief description of the disease in tissue and of the organism producing the disease.

In tissue sections of adult livers, the organisms are found in small (20 to 50 μ) discrete foci throughout the entire liver; in diffuse (150 to 500 μ) generalized areas; in heavy masses involving up to 85 per cent of the liver; or in combinations of the first two types of lesion. Tissue smears of such infected livers, when stained by the Ziehl-Neelsen or Kinyoun method, showed well-stained, discrete, acid-fast bacilli, although it is the opinion of members of this laboratory that the hot method of Ziehl-Neelsen is superior to the Kinyoun method for staining these fish organisms in smears.

The stomach, caeca, and mesenteries of the juvenile fish with the spontaneous infection showed the greatest involvement. Livers were affected to a lesser degree than those of the

TABLE 1

PROTOCOLS EMPLOYED FOR THE TREATMENT OF PREPARED SLIDES

Ziehl-Neelsen Method	Fite-Faraco Method	Johnstone Methods
1. Deparaffinize and hydrate 2. Wash well in running tap water 3. Pretreat*, if any, and wash 4. Stain† in cold carbolfuchsin 5. Destain‡ 6. Wash in running tap water 7. Dehydrate through 95 per cent alcohol 8. Counterstain§ 9. Dehydrate, clear, and mount	1. Deparaffinize in a 1:2 mixture of mineral oil and xylene 2. Blot dry and then wash under tap water 3. Pretreat,* if any, and wash again 4. Stain† in cold carbolfuchsin 5. Destain‡ 6. Wash in running tap water 7. Counterstain,§ if any 8. Blot and let air dry 9. Mount	The various methods employed by Johnstone for staining infected cod tissue were: *a.* Stain 5 minutes in hot carbolfuchsin. Destain for 10 minutes in 20% sulfuric acid; *b.* Stain 10 minutes in hot carbolfuchsin. Destain for 24 hours in 25% sulfuric acid; *c.* Stain 24 hours in cold carbolfuchsin. Destain for 10 minutes in 20% sulfuric acid; *d.* Stain 24 hours in cold carbolfuchsin. Destain for 24 hours in 25% sulfuric acid.

* Pretreatment: *a*) absolute alcohol; *b*) glycerol; *c*) 6% sulfuric acid.
† Stain: 10 ml. 10% alcoholic basic fuchsin in 100 ml. 5% aqueous phenol.
‡ Destain: *a*) 1% alcoholic hydrochloric acid; *b*) 3% aqueous sulfuric acid.
§ Counterstain: *a*) no counterstain; *b*) 1% aqueous Bismark brown; *c*) 0.1% methylene blue.

adult, but showed somewhat the same picture. Tissue infection of the experimentally fed fish was light compared with the other tissues used. Again, however, the bacilli preferred connective tissue elements such as the peritoneal lining adjacent to the kidney. Acid-fast organisms were lightly scattered throughout the kidney and the liver.

The acid-fast organism is a straight rod, 1.5 to 2.5 μ long and 0.5 μ wide, rarely having more than two granules, and the granules are wider than the body of the organism. The bacilli stain with a dark red, uniform color.

The human lung was heavily infected with typical acid-fast bacilli of *Mycobacterium tuberculosis* var. *hominis* that stained well in all modifications.

In order to facilitate the presentation of results obtained from this experiment, the material will be divided into two main groups: I. Fite-Faraco, and II. Ziehl-Neelsen, with three subgroups: human tissue, adult salmon tissue, and juvenile salmon tissue. A summation of modifications and a graphic representation of results are demonstrated in table 2.

I. Fite-Faraco

Human tissue: The organisms in the human tissue stained well in all modifications with apparently little difference in results. The fact that these results were uniformly good substantiates its use as the control.

Adult salmon tissue: Less than 1 to 50 per cent of the organisms were stained in adult salmon tissue that was decolorized with 1 per cent hydrochloric acid. The best results were obtained in those methods that did not include absolute alcohol.

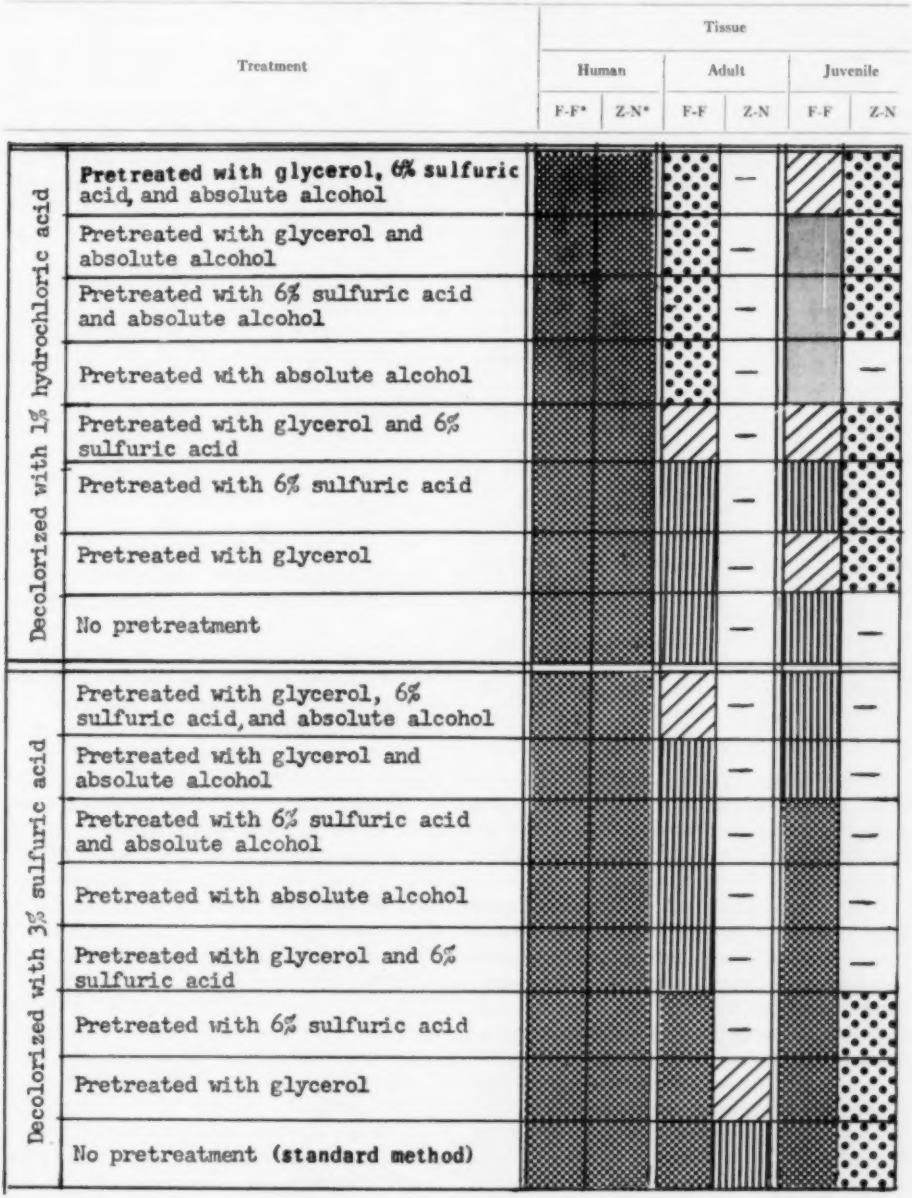
Decolorizing the adult tissue with 3 per cent sulfuric acid resulted in better staining, 10 to 100 per cent of the organisms retaining the dye. Again, those methods from which absolute alcohol was excluded were the most successful. The method in which no pretreatment was applied was used in this latter group, and is the standard technique employed in this laboratory. Two other methods that obtained 100 per cent results were pretreatment with glycerol and pretreatment with 6 per cent sulfuric acid.

The difference between decolorizing with hydrochloric acid and decolorizing with sulfuric acid can be more readily appreciated when scanning a slide. Areas of organisms were more readily detected in the sulfuric-acid decolorized groups than in the hydrochloric-acid groups. Sulfuric acid darkened the color of the organisms and removed a greater amount of acid-fuchsin dye from the tissue than did the hydrochloric acid.

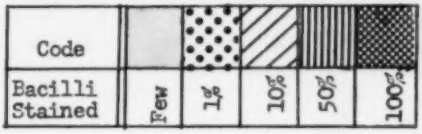
Counterstaining modifications presented equally distinct results. The Bismark brown coun-

TABLE 2

A Comparison of the Staining Characteristics of Acid-fast Organisms in Adult and Juvenile Chinook Salmon Tissue (*Oncorhynchus tshawytscha*) and Human Tissue when Stained by the Fite-Faraco and Ziehl-Neelsen Methods and Counterstained with Methylene Blue



* F-F Fite-Faraco method
Z-N Ziehl-Neelsen method



terstain tended to mask the organisms on all slides except those that had no pretreatment and were decolorized by the sulfuric acid. For this reason it was discarded as a counterstain. In noncounterstained tissue the areas of organisms were not sufficiently differentiated from the surrounding cells to enable quick, positive detection and identification. There was some masking by the methylene blue counterstain, but the effect was limited and the differentiation of infected areas was excellent, as was the differentiation of the individual organisms.

Juvenile salmon tissue: Results obtained with the experimentally and spontaneously infected juvenile fish were essentially the same. All tissues, even those which were pretreated with absolute alcohol, stained better than the adult tissues. In these juvenile fish, the glycerol pretreatment may have exerted a protective action on the organisms, since the organisms in tissue pretreated with this reagent were stained better and the number of stained bacteria was greater. This fortunate effect seemed to be especially true in the group that was decolorized by sulfuric acid in which the number stained ranged from 50 to 100 per cent. However, the difference is so slight and questionable, it must be discounted. In general, the sulfuric acid-decolorized group was stained much better than the group decolorized with hydrochloric acid.

II. Ziehl-Neelsen

Human tissue: The organisms in the human tissue stained well in all modifications and, as with the Fite-Faraco method, there was apparently little difference between the various changes.

Adult salmon tissue: All of the adult salmon tissue decolorized by hydrochloric acid appeared negative for acid-fast organisms when stained by the Ziehl-Neelsen method. Occasionally there were areas that had taken up the acid-fast stain, but no distinctly identifiable organisms could be detected.

In the sulfuric-acid decolorized group only those tissues pretreated with glycerol or non-pretreated were positive. The glycerol-treated tissue had about 10 per cent of the organisms stained, and these were all distinct, well-stained rods. The non-pretreated or standard method showed 50 per cent of the organisms stained.

Juvenile salmon tissue: There was little difference in the response of the experimentally and spontaneously infected fish, which was in agreement with the response to the Fite-Faraco method.

In the hydrochloric-acid decolorized group, few organisms were stained by the Ziehl-Neelsen method. Two groups, those pretreated exclusively with absolute alcohol and those that did not receive any pretreatment, appeared to be negative. The response elicited by the other methods was light, less than 1 per cent, but far better than in the adult salmon tissue, in which it was entirely negative.

Decolorization of the Ziehl-Neelsen-stained organisms with 3 per cent sulfuric acid appeared to be too strong a process because only three methods of treatment gave positive results to the extent of about 1 per cent of the organisms being stained. All other tissues were negative. This was in contrast to the hydrochloric-acid decolorization in which all tissues but the non-pretreated and absolute-alcohol pretreated tissue were positive.

Discussion

Little difficulty was encountered in staining the acid-fast organisms in tissue smears, although the hot Ziehl-Neelsen method was preferred to the cold Kinyoun method. This preference was due to the fact that tissues having few organisms were often considered negative when stained by the cold method.

One of the major obstacles that had to be overcome in this experimental work was the affinity of the fish tissue for the carbolfuchsin stain. Some of the methods, such as the Kinyoun, hot Ziehl-Neelsen, and all of the Johnstone techniques, stained the tissue to such an extent that the dye could not be removed sufficiently to differentiate some of the host-cell characteristics.

The agents used for pretreatment were selected for a specific purpose in each case. The glycerol was used to act as a possible protective agent, the 6 per cent sulfuric acid as an oxidizing agent, and the absolute alcohol as a possible deterrent to the staining of the organism through lipid extraction.

Without question, the Fite-Faraco method proved to be the best method for staining the bacilli observed in the tissue of salmonoid fishes. The results, including all possible variations in pretreating, destaining, and counterstaining, were positive. The bacilli usually stained a uniformly bright to deep red color. All sections

destained with 3 per cent sulfuric acid were distinctly positive regardless of the additional modifications in procedure.

Very poor results were obtained with the Ziehl-Neelsen method of staining. Sections of adult salmon liver when stained by this method and decolorized by 1 per cent hydrochloric acid were negative, even though identical material stained by the Fite-Faraco method was poorly to strongly positive. In this same tissue, when the Ziehl-Neelsen–3 per cent sulfuric acid technique was used, only two tissues, those pretreated with glycerol and with the standard method, were positive.

The absolute alcohol seemed to fulfill its function of removing lipids. With the exception of human tubercle bacilli, all tissues treated with this reagent appeared negative or poorly positive. When used in combination with 6 per cent sulfuric acid in the Ziehl-Neelsen method, there was a marked increase in the number of fragmented organisms observed in sections known to be strongly positive when stained by the Fite-Faraco method. These fragments did not stain at all and were indistinguishable from host-cell debris.

Organisms that stained when treated with 6 per cent sulfuric acid were much darker in color, almost purple, than were the stained organisms in the non-pretreated or other pretreated tissue. However, the improvement in staining was not sufficiently better than the standard method, decolorized with sulfuric acid, to warrant its retention as an extra step in the routine procedure.

Glycerol appeared to have slight or no effect on the staining of the acid-fast organism of the fish.

It was hoped that, by not counterstaining the tissue, more of the organisms could be detected than in tissue counterstained with Bismark brown or methylene blue. It was far more difficult, however, to identify areas of infection in the non-counterstained tissues than in the counterstained ones, and the organisms were less distinct.

Bismark brown was used in an attempt to cover the red color of the host cells resulting from staining with the carbolfuchsin. The brown stain did not produce this result and, in addition, it tended to mask the organisms and thus rendered them less distinct and identifiable.

Methylene blue proved to be the counterstain of choice, since it contrasted the areas of acid-fast organisms, did not mask the bacteria to the extent that the Bismark brown did, and consequently rendered the organisms more distinct and readily identifiable.

Organisms in the juvenile chinook salmon also responded poorly to the Ziehl-Neelsen method; however, the results were somewhat better than for the adult tissue. Usually a few bacilli could be found in the tissues which were strongly positive by the Fite-Faraco method. Sulfuric acid decolorization did not have a salutary effect; indeed, it appeared to reduce the number of acid-fast organisms.

The observation that the acid-fast organism in the young salmon tissue stained better than that in the adult salmon tissue was thought to be due to the presence of a greater amount of oils or of specific oils in the tissues of the juvenile fish. This factor was considered before the experimental work was started and was the cause of much concern in selecting an oil for the Fite-Faraco method, since there was a possibility that the oil itself might enter directly into the staining reaction. For this reason, mineral oil (which is not acid fast) was chosen. The choice proved to be a fortunate one, and the oil is still being used in the routine staining of suspected material. In passing, it may be noted that Solomidès (17, 18) used both peanut and mineral oil in *in vitro* growth of human and bovine tubercle bacilli and obtained growth that was predominantly nonacid fast.

Excellent results were obtained with sections from human lung tissue. All modifications were positive, the sulfuric acid decolorization again tending to darken the staining reaction.

Results using the Johnstone modifications were entirely negative. The tissue retained the stain and could not be destained by any method.

Decalcification of the whole, juvenile fish with 5 per cent nitric acid did not seem to have any effect on the staining properties of the organism. Sections of adult liver that were processed simultaneously as a control gave the same staining reactions as the non-nitric acid–treated adult sections. Extended preservation in buffered formalin apparently did not alter the staining characteristics of the bacilli since the organisms in both the fresh, experimentally infected fish tissue and the tissue preserved for twenty-four months stained in the same way.

Summary

Modifications of the standard Ziehl-Neelsen and Fite-Faraco methods have been attempted. Under the present experimental conditions, the Ziehl-Neelsen method has proved to be unsatisfactory for staining acid-fast organisms in salmonoid fish tissue sections. The Fite-Faraco method of deparaffinizing sections with a mixture of "light" oil and xylene was modified by the use of "extra heavy" mineral oil, decolorizing with 3 per cent sulfuric acid, and counterstaining with 0.1 per cent methylene blue. The modified Fite-Faraco technique is the method of choice for staining these organisms.

A comparison of the staining characteristics of human tubercle bacilli and the acid-fast organism observed in fish tissue indicates that differences between the two are sufficient to preclude any attempt to equate them.

A difference in the staining characteristics of the acid-fast bacillus in adult and juvenile chinook salmon (*Oncorhynchus tshawytscha*) has been noted and an explanation was postulated on the basis of differences in oil content of the two groups. Attention has been brought to the observation that standard methods of staining may not be the best for detection of atypical organisms in tissue sections.

Sumario

Estudio Comparativo de una Enfermedad Micobacteriana de los Peces Salmonoides. I. Comparación de las Características Colorantes de la Enfermedad de los Peces con las de la Tuberculosis Humana en Cortes Teñidos con las Técnicas de Fite-Faraco y Ziehl-Neelsen

Se han ensayado modificaciones de los métodos aceptados de Ziehl-Neelsen y Fite-Faraco. En las actuales condiciones de experimentación, el método de Ziehl-Neelsen resultó poco satisfactorio para teñir microbios ácidorresistentes en cortes de tejidos de peces salmonoides. El método de Fite-Faraco de desparafinizar los cortes con una mezcla de aceite "liviano" y xilol fué modificado mediante el empleo de aceite mineral "extrapesado", descolorando con 3 por ciento de ácido sulfúrico y contracoloreando con 0.1 por ciento de azul de metileno. La técnica de Fite-Faraco modificada es el método de elección para la coloración de dichos microbios.

Una comparación de las características colorantes del microbio tuberculoso humano y del microbio ácidorresistente observado en los tejidos de pez indica que las diferencias entre ambos bastan para vedar todo esfuerzo encaminado a equipararlos.

Se ha notado una diferencia en las características colorantes del bacilo ácidorresistente entre el salmón "chinook" (*Oncorhynchus tshawytscha*) adulto y el juvenil, postulándose una explicación a base de la diferencia en el contenido de aceite en los dos grupos. Se llama la atención sobre la observación de que los métodos normalizados de coloración quizás no sean los mejores para el descubrimiento de microbios atípicos en los cortes histológicos.

Resumé

Étude comparative de l'agent responsable d'une maladie mycobactérienne chez les poissons salmonoïdes. I. Comparaison des caractéristiques de coloration de la maladie du poisson et de la tuberculose de l'homme dans des coupes colorées par les méthodes de Fite-Faraco et de Ziehl-Neelsen

Il a été tenté de modifier les méthodes standard Ziehl-Neelsen et Fite-Faraco. Dans les conditions expérimentales présentes, la méthode Ziehl-Neelsen ne s'est pas montrée satisfaisante pour la coloration des organismes acido-résistants dans les coupes de tissus de poissons salmonoïdes. La méthode Fite-Faraco de déparaffinisation des coupes par un mélange d'huile "légère" et de xylène a été modifiée par l'emploi d'huile minérale "extra-lourde" en décolorant avec l'acide sulfurique à 3%. et en recolorant avec le bleu de méthylène à 0,1%. La technique modifiée Fite-Faraco est la méthode de choix pour la coloration de ces organismes.

Une confrontation des caractéristiques de coloration des bacilles tuberculeux humains et des microorganismes acido-résistants dans les tissus du poisson indique que leurs différences sont suffisantes pour prévenir tout essai de comparaison.

Les auteurs ont observé une différence dans les charactéristiques de coloration du bacille acido-résistant chez les saumons chinook adultes ou jeunes (*Oncorhynchus tshawytscha*) et ils ont proposé une explication basée sur les différences de la teneur en huile des deux groupes. L'attention a été attirée sur le fait que les méthodes standard de coloration pourraient ne pas être les meilleures pour déceler les microorganismes atypiques dans les coupes de tissu.

Acknowledgment

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CATALASE AND PEROXIDASE IN MYCOBACTERIA[1, 2]

Possible Relationship to the Mode of Action of Isoniazid

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Introduction

The fact that there is a general correlation between isoniazid susceptibility and catalase (1, 2) and peroxidase (3, 4) activity in *Mycobacterium tuberculosis* has led to a belief that one or the other of these enzymes plays a part in sensitizing the bacteria to isoniazid. Several hypotheses have been put forward to explain the connection (5, 6). This has resulted in a renewed interest in mycobacterial catalase and peroxidase, and several recent studies on these enzymes have been published (3, 4, 7–12). The results in the present paper supplement these studies. A summary of some of these results has been published (13), but it is now clear that peroxidase values given there are too high.

The work described in this paper was also done in the hope of finding some explanation of how mycobacterial catalase, peroxidase, or some substance connected with their biosynthesis, might bring about the antituberculous action of isoniazid. While no definite explanation of this emerged, some evidence was obtained which supports the present writer's hypothesis that isoniazid works through the formation of free radicals (probably hydroxyl radicals) from its metal-catalyzed autoxidation.

Materials and Methods

Organisms studied: Mycobacterium tuberculosis BCG (NCTC 5692) and *M. phlei* (NCTC 525) were obtained from the National Collection of Type Cultures, Colindale Avenue, London. *M. smegmatis* was a strain maintained in these laboratories for a number of years. The isoniazid-resistant strain of *M. smegmatis* was obtained by serial culture in isoniazid-containing Proskauer and Beck medium (14) containing 5 per cent human serum until it was resistant to 1 mg. of isoniazid per ml. This strain maintained its resistance on repeated subculture in the absence of isoniazid.

Media: The BCG was maintained on Löwenstein-Jensen medium. *M. smegmatis* and *M. phlei* were maintained on Proskauer and Beck medium, which in the case of isoniazid-resistant *M. smegmatis* contained 5 per cent human serum and 0.5 mg. of isoniazid per ml.

The cultures of these organisms for the experiments were grown on Löwenstein-Jensen medium or on Proskauer and Beck medium, which was usually metal-supplemented as described in the text.

Preparation of suspensions for enzyme studies: The organisms were suspended, usually in 0.05 M phosphate buffer, pH 7.0, by grinding for the minimal necessary time in a slowly rotating ball-mill. The suspension was then diluted with the buffer to the desired turbidity (usually to give 1.6 mg., dry weight, per ml.). The suspension was then centrifuged, the supernatant removed, and the cells were resuspended to the same turbidity. The cells were disrupted, after a preliminary chilling, by shaking in a Mickle disintegrator (for ten to fifteen minutes for *M. smegmatis* and *M. phlei*, twenty to thirty minutes for *M. tuberculosis*), with ballotini beads, No. 12.

Fractional precipitation of cell-free extracts with ethanol: A suspension of the bacilli (about 10 mg., dry weight, per ml.) in 0.1 M phosphate buffer, pH 7.0, was shaken in the Mickle disintegrator for thirty minutes. The suspension was then centrifuged at 3,000 g. for ten minutes; the supernatant was chilled and precipitated successively with 1, 1.5, 2, 3, and 5 volumes of ice-cold ethanol for fourteen, three, three, three, and fourteen hours, respectively, at 0°C. The precipitates were suspended in 0.05 M phosphate buffer, pH 7.0, and the solutions were clarified by centrifuging.

Determination of catalase activity: One milliliter of water (or 1 ml. of the desired buffer, about 0.05 M, when the suspension was unbuffered) and 1 ml. of bacterial or enzyme suspension were pipetted into a test tube in a bath at about 18°C. One milliliter of 100 γ of hydrogen peroxide per ml. solution was run in from a pipette, and the reaction was stopped after exactly two minutes by pipetting in 1 ml. of titanium sulfate reagent (15*a*). The tube was centrifuged and the extinction read at 400 mμ. Blanks and zero minute peroxide determinations were also done.

If stronger buffers than those recommended are used, a basic titanium salt may be precipitated. Nitrite is one of the very few substances which interfere with the method of determining peroxide.

The specific catalase activity of the preparation was calculated from the equation:

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[1] From the Laboratories of the Medical Research Council of Ireland, Trinity College, Dublin, Ireland.

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specific catalase activity

$$= \frac{2.3}{t \times c} \log \frac{x_0}{x_t} \text{ (L. } \times \text{ gm.}^{-1} \times \text{ min.}^{-1})$$

where t is the reaction time in minutes, c is the concentration of cell material or other enzyme preparation in grams dry weight per liter of the reaction mixture, x_0 is the concentration of hydrogen peroxide at the start of the reaction, x_t is the final concentration of hydrogen peroxide. The specific activity may be converted to the *Kat. f.* value by multiplying by $\frac{20}{2.3}$.

Owing to enzyme inactivation, the specific activity decreases during the course of catalase action, and a true value can be obtained only by determination after several time intervals and extrapolating to zero time. However, the value after two minutes is a reasonable approximation of the value at zero time.

Determination of peroxidase activity: Since mycobacterial peroxidase has no effect on guaiacol, the rather unsatisfactory substrates of pyrogallol or catechol were used.

One milliliter of water or the desired buffer, 1 ml. of the bacterial or enzyme suspension, and 1 ml. of 1 per cent pyrogallol were pipetted into a 1-cm. cell of a Beckman spectrophotometer, mixed rapidly, and the cell was placed in the spectrophotometer (the cell compartment being maintained at about 18°C. by circulating water). One-hundredth to two-hundredths of a milliliter of 1 per cent hydrogen peroxide (the actual amount being judged from the catalase activity of the preparation) was added on the end of a stirring rod, and the mixture was stirred rapidly. The extinction at 430 mμ was read and a stop-watch was started at the same time. The extinction was read every minute until a maximal rate of increase was reached. Exactly the same experiment was then performed without the addition of hydrogen peroxide.

The maximal rate of increase of extinction less the rate of increase of extinction in the absence of hydrogen peroxide was taken to be directly proportional to the peroxidase activity. This was shown to be approximately true for solutions of horse-radish peroxidase,[2] diluted to the activities encountered with the mycobacterial preparations. The results were standardised by carrying out a purpurogallin number (PZ) determination by the Willstatter and Stoll procedure (16) on some of the preparations and using the results to convert all of the activities to PZ values.

The results were not truly quantitative owing to the accompanying catalase activity and, possibly, to other interfering substances in the crude extracts.

Results

Catalase Activities of Some Mycobacteria

Most of these studies were made on mycobacteria grown on a medium not containing Tween® 80. Hence, in order to obtain cell suspensions suitable for quantitative work, it was necessary to grind the material taken from the surface of the medium. A rotating ball-mill, containing agate balls, was found to give the most uniform suspensions. These suspensions were then centrifuged to remove traces of the culture medium, and the cells were resuspended in fresh buffer by sucking up several times in a Pasteur pipette. The supernatant from the centrifuging was found to contain some catalase. This was first thought to be extracellular catalase, readily eluted from the cells during grinding. Further investigation showed that most, and probably all, of the catalase in the supernatant arose as a result of cell damage during grinding. Hence, this grinding was continued for the shortest period necessary to give a smooth suspension, and the catalase level of the supernatant was checked to confirm that it was low.

Because of permeability problems (to be dealt with later), it was necessary to rupture the bacterial cells in a Mickle disintegrator, as described earlier, in order to determine the total catalase level. All of the catalase activity was found in solution when the cells were ruptured, leaving the cell debris inactive.

The specific activities of actively growing cultures of BCG on Proskauer and Beck medium, on Löwenstein-Jensen medium, or in Tween-albumin Proskauer and Beck medium fell in the range 2.2 to 3.5 L. $\times$ gm.$^{-1}$ $\times$ min.$^{-1}$.

A decrease in catalase activity with increasing age of the culture was observed, but this decrease was rather irregular and did not become considerable until the viability began to drop. Most of the decrease appeared to be due to a decrease in the percentage of viable organisms in the culture, rather than to a decrease in the catalase per viable cell. In very old cultures of low viability the catalase activity was practically zero.

The precise composition of the medium had little effect on the catalase content of BCG while the cells were actively growing but, as the cultures aged, those growing on media supplemented with iron, serum, or particularly with hemin maintained their catalase levels much better than those not so supplied. This was particularly noticeable with Proskauer and Beck medium, in which the level of trace elements is low and in which a deficiency, particularly of iron and zinc, developed when growth became heavy. Precise

[2] A product of Nutritional Biochemicals.

studies were not carried out to see how much of the effect of added metals was due to a direct effect on catalase synthesis and how much to an effect on viability. Bönicke (12) found a more regular variation of the catalase activity of H37Rv on Löwenstein-Jensen medium with age, and with the presence or absence of hemin, than was shown by BCG in the above experiments.

Actively growing cultures of *M. smegmatis* had activities varying from 0.2 to 3.0 L. × gm.$^{-1}$ × min.$^{-1}$, thus showing a much greater range of variation than BCG. The catalase activity was controlled by the content of certain metals in the medium (table 1). Iron, zinc, and cobalt were required for high levels of catalase with a medium impoverished in metals. These metals added singly or in pairs had little effect or even decreased the catalase, except for the combination of iron and zinc. Thus, iron and zinc together play the major role in producing a high catalase, cobalt producing a slight further increase. Other metals tested had no clear-cut effect in increasing the catalase further. These effects were strikingly similar to the effects of the same metals on the desoxyribonucleic acid content of this organism (15*b*). The effect of these metals on various aspects of the metabolism of mycobacteria is being investigated at present in these laboratories, and it is too soon to say whether the requirement is specifically for catalase, peroxidase, and desoxyribonucleic acid synthesis, or whether the low levels of these substances result from a distortion of metabolism elsewhere.

A consistent variation with the age of the culture was not observed. Very old cultures did not show the very low levels of activity observed with very old BCG, probably because the viability remained higher.

As has already been reported (2), isoniazid-resistant strains of *M. smegmatis* have normal catalase activity when grown in the absence of isoniazid. When harvested from media containing 500 γ of isoniazid per ml., however, a strain resistant to 1 mg. per ml. had a catalase activity of only 0.2 L. × gm.$^{-1}$ × min.$^{-1}$, despite an adequate supply of trace metals. This was probably due to the inhibition of the catalase by exposure to the isoniazid (which will be dealt with later), and it is possible that the fact that *M. smegmatis* cannot become resistant to more than about 1 mg. per ml. is due to complete catalase destruction at higher isoniazid concentrations, combined with peroxide formation from isoniazid in the medium (17).

TABLE 1

EFFECT OF METALS IN GROWTH MEDIUM ON CATALASE ACTIVITY OF M. SMEGMATIS

Growth Medium	Catalase Activity of Ruptured Cells
	L. × gm.$^{-1}$ × min.$^{-1}$
Basal	0.52
Basal + 2.0 γ/ml. Fe^{+++}	0.64
Basal + 0.4 γ/ml. Zn^{++}	0.27
Basal + 0.05 γ/ml. Co^{++}	0.20
Basal + Fe^{+++} + Co^{++}	0.53
Basal + Zn^{++} + Co^{++}	0.51
Basal + Fe^{+++} + Zn^{++}	1.7
Basal + Fe^{+++} + Zn^{++} + Co^{++}	2.1

The cultures were six days old, and the basal medium was Proskauer and Beck medium autoclaved with 1 per cent alumina to remove trace metals.

Fe—iron
Zn—zinc
Co—cobalt

The few cultures of *M. phlei* examined had catalase activities close to 1.7 L. × gm.$^{-1}$ × min.$^{-1}$.

The catalase activities recorded above, when converted to *Kat.f.* values, give higher results than those obtained by Finlayson and Edson (7) and Andrejew and associates (9), even when allowance is made for the fact that they used suspensions of unruptured cells. Much of this difference is due to the fact that their determinations were done at 0°C.

Catalase Activities of Unruptured Cells: Permeability to Hydrogen Peroxide

The catalase activity of suspensions of *M. smegmatis* was increased by one half at the most on rupture of the cells. Hence, either most of the catalase is located on the cell surface or hydrogen peroxide penetrates into the cells at a rate comparable with the rate at which it is destroyed by catalase. If the enzyme were located on the cell surface, its activity would be affected by the external pH in the same way as the catalase in a cell-free extract. That this was not the case is shown in table 2; hence, the greater part of the enzyme must be located within the cell. Cells of *M. smegmatis* must therefore be freely permeable to hydrogen peroxide.

Whole cells of BCG showed about one-fifth of the catalase activity of ruptured cells, and this fifth was mainly surface catalase accessible to changes of external pH (table 2). Therefore, most of the catalase in whole cells of this organism is inaccessible to external hydrogen peroxide (as has been found by other workers also (8)), suggesting that the cell membrane of BCG is relatively impermeable to hydrogen peroxide. This theory was supported by incubating a suspension of BCG (0.4 mg., dry weight, per ml.) with 25 γ of hydrogen peroxide per ml. for eighteen hours. After this period the surface catalase was reduced in quantity, but the internal catalase was unaffected. A combination of surface catalase with relative impermeability to hydrogen peroxide would provide BCG with an effective protection against external catalase. Catalase-negative strains presumably also have a low permeability to hydrogen peroxide but, in the absence of catalase, enough can penetrate from low levels in the external medium to kill the cells (15*a*).

M. phlei was intermediate between *M. smegmatis* and BCG in its apparent permeability to hydrogen peroxide.

M. tuberculosis is not unique in being apparently poorly penetrated by hydrogen peroxide. For example, the catalase in the large granule fraction of liver is virtually inaccessible to hydrogen peroxide until the granules are disrupted (*see* 46).

TABLE 2

EFFECT OF pH ON THE CATALASE ACTIVITY OF SUSPENSIONS OF WHOLE AND RUPTURED CELLS OF M. SMEGMATIS AND BCG

pH	*M. Smegmatis*		BCG	
	Catalase Activity at Optimal pH L. × gm.$^{-1}$ × min.$^{-1}$			
	Whole 1.42	Ruptured 1.90	Whole 0.42	Ruptured 2.91
	Catalase Activity Per Cent of Activity at Optimal pH			
	Whole	Ruptured	Whole	Ruptured
6.0	95	51	60	41
6.5	97	72	75	66
7.0	100	98	91	100
7.5	100	100	100	100
8.0	97	71	86	60

Peroxidase Activities of Some Mycobacteria

Using the method described earlier, the following PZ values were obtained for suspensions of ruptured cells: BCG, 0.14–0.36; *M. smegmatis*, 0.034–0.20; *M. phlei*, 0.17. These results are somewhat lower than those of Andrejew and associates (3).

The peroxidase activities of these mycobacteria were affected by the same factors as those which affected catalase activities. The ratio of catalase to peroxidase activity was surprisingly close to constancy in each strain, although there were slight variations. It is tempting to suggest that a constant ratio does exist in each strain, and that the deviations are due to inaccuracies in the peroxidase assay caused by the presence of catalase and other interfering substances, such as reducing agents. This has a bearing on the possibility that the catalase and peroxidase are the one enzyme, differing slightly in properties from strain to strain.

Compared with most peroxidases, the mycobacterial enzyme acted on a very restricted range of hydrogen donors. It oxidized dihydroxyphenols and polyhydroxyphenols, such as catechol, hydroquinone, and pyrogallol, but not monohydroxyphenols, guaiacol, aromatic amines, or uric acid. The ratio of the rate of oxidation of pyrogallol to the rate of oxidation of catechol was approximately the same for this enzyme as for horse radish peroxidase. No distinct difference in the range of hydrogen donors oxidized was observed between the peroxidase from BCG and that from *M. smegmatis*.

Possible Identity of Mycobacterial Catalase with Peroxidase

The possible constancy of the ratio of catalase to peroxidase in mycobacteria suggests the possibility that these are two functions of the same enzyme. The general correlation between catalase and peroxidase in isoniazid-resistant strains of *M. tuberculosis* led Hedgecock and Faucher (4) to make the same suggestion. This contention is not easy to prove, however. The evidence given below tends to support it, without giving decisive proof.

When cell-free extracts of *M. smegmatis* were fractionated with ethanol, as described earlier, most of the catalase and peroxidase activities were found in the fraction precipitated by three volumes of ethanol, with smaller amounts in the

fractions precipitated by two volumes and five volumes. All of these fractions showed the same catalase to peroxidase ratio as the original extract. This is not a strong argument in favor of the identity of these enzymes, as the separation of peroxidase from catalase is often difficult. When attempts were made to fractionate extracts of BCG in the same way, practically all of the activity of both enzymes was lost, apparently due to their inactivation by ethanol.

The pH optimum of the catalase of BCG and *M. smegmatis* was between 7.0 and 7.5, and that of the peroxidase about 7.5. The peroxidase optimum could not be determined accurately, however, as autoxidation of pyrogallol became rapid above pH 7.0, and it was difficult to allow for the effect of oxygen formed by the catalase action. Most peroxidases are rather heat stable (myeloperoxidase is unaffected by 60°C. for ten minutes, and horse-radish peroxidase by 63°C. for fifteen minutes (18)), while catalases are rather heat labile (spinach catalase is completely inactivated by 60°C. for ten minutes (18)). Nevertheless, the two activities were lost to a similar degree when extracts of mycobacteria were heated to 60°C. for ten minutes (table 3). Both activities were considerably more labile in BCG than *M. smegmatis*.

Catalase is very sensitive to azide, a substance that normally has no effect on peroxidases at pH 7.0. Both activities in mycobacterial extracts were affected by azide, though there were apparent differences in degree of inhibition (table 3).

TABLE 3

COMPARISON OF SOME PROPERTIES OF THE CATALASE AND PEROXIDASE IN EXTRACTS OF BCG AND M. SMEGMATIS

	BCG		*M. Smegmatis*	
	Catalase	Peroxidase	Catalase	Peroxidase
Optimal pH	7.0-7.5	About 7.5	7.0-7.5	About 7.5
	per cent	*per cent*	*per cent*	*per cent*
Inactivation on heating at 60°C. for 10 min. at pH 7.0	99.3	98.5	65	83
Inhibition by NaN_3 at pH 7.0				
2×10^{-4} M NaN_3			97	88
0.5×10^{-4} M NaN_3	87	31	92	80

NaN_3 = sodium azide

Thus, the catalase and peroxidase in the two strains examined agreed more closely in their properties than do other catalases and peroxidases. Their properties appeared not to be identical, however, and explanations of the divergencies would have to be found before it could be concluded definitely that they are the same enzyme. Further purification, followed by a comparison of their physical properties, should permit a definite decision to be reached. A direct proof of this identity was sought by showing that provision of a hydrogen donor for the peroxidase activity reduced the catalase activity, as shown by formation of oxygen from peroxide. However, the donors tested (catechol and pyrogallol) at the levels used (200 γ per ml.) also inhibited beef-liver catalase, although this has no peroxidase activity.

Effect of Isoniazid on Mycobacterial Catalase and Peroxidase

As has been reported (2, 8), isoniazid inhibited mycobacterial catalase. The degree of inhibition depended on the species of Mycobacterium (*M. tuberculosis* catalase being particularly susceptible), on the concentration of isoniazid, on the length of exposure of the enzyme to the isoniazid, on the temperature at which this exposure took place, on the presence of oxygen, and on the metal ions present (table 4). Isoniazid has probably no effect in the absence of metal ions. Copper was considerably the most active metal in catalyzing the action of isoniazid on catalase. The catalase was more rapidly inactivated by isoniazid which had been incubated with Cu^{++} for some time than when the two solutions had been freshly mixed.

These results may best be explained by the hypothesis that inhibition is caused, at any rate at lower concentrations of isoniazid, not by the combination of isoniazid with the heme groups of the catalase, but as a consequence of the metal-catalyzed autoxidation of isoniazid. Chance (*see* 45) has found that catalase is inactivated through the formation of inactive catalase-hydrogen peroxide complex II when catalase is exposed to continuously generated hydrogen peroxide, such as is formed by autoxidation of ascorbic acid. However, the inactivation of mycobacterial catalase by isoniazid autoxidation does not seem to be due to complex II formation for the following reasons: (*1*) Although manganese, Mn^{++}, catalyzed the autoxidation of isoniazid more rapidly than Cu^{++} under the

TABLE 4

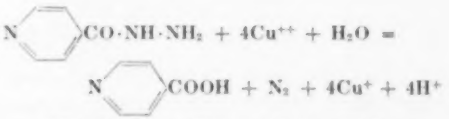
EFFECT OF ISONIAZID ON CATALASE ACTIVITY OF CELL-FREE EXTRACTS OF BCG AND M. SMEGMATIS

Additions to Extract	BCG				*M. Smegmatis*	
	Period of Exposure before Catalase Determination					
	Hours					
	0	3	6	10	0	3
	Per Cent Inhibition					
Incubation at 0°C.						
3 γ of isoniazid per ml.		2.5				1.8
13 γ of isoniazid per ml.		5.5				3.9
Incubation at 18°C						
3 γ of isoniazid per ml.	0.0	3.0	7.5	10.0	0.0	2.8
3 γ of isoniazid per ml. + 0.5 γ of Cu^{++} per ml.			19.0	33.0		
3 γ of isoniazid per ml. + 0.5 γ of Cu^{++} per ml. (partially anaerobic)			10.0			
0.5 γ of Cu^{++} per ml.			6.0			
15 γ of isoniazid per ml.	0.0	13.0			0.0	7.4
2.5 γ of Cu^{++} per ml.		5.0				
30 γ of isoniazid per ml.	1.9	15.0				
30 γ of isoniazid per ml. + 2.5 γ of Cu^{++} per ml.	30.0	70.0				
30 γ of isoniazid per ml. + 2.5 γ of Cu^{++} per ml. (extract added after 3-hour incubation of isoniazid and copper)	52.0					
30 γ of isoniazid per ml. + 10 γ of Cu^{++} per ml.	59.0	96.0				
30 γ of isoniazid per ml. + 10 γ of Mn^{++} per ml.		45.0				
30 γ of isoniazid per ml. + 10 γ of Fe^{+++} per ml.		18.0				
30 γ of isoniazid per ml. + 10 γ of Zn^{++} per ml.		12.0				
30 γ of isoniazid per ml. + 10 γ of Co^{++} per ml.		13.0				
30 γ of isoniazid per ml. + 10 γ of hemin per ml.		44.0				
10 γ of hemin per ml.		16				
Incubation at 37°C.						
3 γ of isoniazid per ml.		10.0				3.0
15 γ of isoniazid per ml.		18.5				9.2

Cu—copper
Mn—manganese
Fe—iron
Zn—zinc
Co—cobalt

conditions of the experiments, with formation of hydrogen peroxide (17), yet the autoxidation catalyzed by Mn^{++} did not inactivate the catalase to the same extent as that catalyzed by Cu^{++} (table 4). (*2*) Ethanol or methanol (0.01 M) (*see* 45) did not reverse, or prevent, the inactivation of mycobacterial catalase caused by isoniazid autoxidation.

It is suggested instead that the inactivation was caused by free radicals formed in the autoxidation of isoniazid. In the copper-catalyzed autoxidation of isoniazid, free radicals, capable of polymerizing styrene for example, appear to be produced (15*b*). These are probably hydroxyl radicals produced by a mechanism such as the following:



$$2Cu^{+} + O_2 + 2H^{+} = 2Cu^{++} + H_2O_2$$

$$Cu^{+} + H_2O_2 = Cu^{++} + OH\cdot + OH^{-}$$

In this mechanism isoniazid functions merely as a reducing agent capable of forming Cu^{+} from Cu^{++}, and the Cu^{+} then forms the destructive free radicals. This is supported by the observation that hydrogen peroxide increased the effect of copper plus isoniazid, which would be predicted from the above equations; and the observation that certain other reducing agents (such as ascorbic acid and a number of hydrazides)inac-

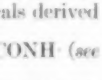
tivated mycobacterial catalase in the presence of Cu^{++}.

Other metal ions, except for Mn^{++}, had little effect on the inactivation of catalase by isoniazid, as they have little effect on the autoxidation of isoniazid (16*b*). Hemin, however, significantly stimulated the inactivation of catalase by isoniazid, as might be expected on the free radical hypothesis.

The catalase of isoniazid-resistant *M. smegmatis* was inhibited to the same extent as that of the susceptible strain. Beef-liver catalase was comparatively insensitive to inhibition by isoniazid.

The effect of isoniazid on mycobacterial peroxidase has not been determined because, in addition to any effect it may have on the enzyme, isoniazid reacts with quinone intermediates formed during the action of the peroxidase on the hydrogen donors. For example, when isoniazid was incubated with horse-radish peroxidase, hydrogen peroxide and hydroquinone, the same brown condensation product was obtained as was formed when isoniazid was incubated with *p*-benzoquinone.

Discussion

Although the inhibition of catalase by high concentrations of isoniazid may be due to the formation of a catalase-isoniazid complex (8), the inhibition by low concentrations of isoniazid results most likely from its autoxidation, probably catalyzed by cupric ions or by a heme compound. The copper- or heme-catalyzed autoxidation probably results in the formation of free radicals derived from oxygen, such as hydroxyl radicals ($OH^{\cdot}$), and also possibly radicals derived from isoniazid itself, such as  (*see* 43). It is suggested that the inhibition of isoniazid is due to attack by these radicals.

It has been accepted that isoniazid combines with the aldehyde group of pyridoxal in the transaminases (19), cysteine desulfhydrase (20), and amino-acid decarboxylases (21); that it competes with diphosphopyridine nucleotide in malic dehydrogenase (22), other dehydrogenases (23), and diphosphopyridine nucleotidases (24); while a variety of explanations has been offered for the inhibition of amine oxidase (25), cytochrome oxidase (26), asparaginase (27), lipase (28), and acetate oxidation enzymes (29). However, in two cases in which the inhibition has been studied in detail (30, 31), the pattern of inhibition is very similar to that of catalase, suggesting that metal-catalyzed autoxidation of isoniazid with free radical formation may be responsible for a number of these inhibitory effects.

The bactericidal effect of isoniazid on *M. tuberculosis* is slow (32), irreversible (33), requires oxygen (34), and is accompanied by a reduction in the amount of isoniazid recoverable (35). This pattern suggests that this bactericidal effect also may be brought about by free radicals produced in the autoxidation of isoniazid. This hypothesis raises a number of questions and difficulties:

(*1*) Why is *M. tuberculosis* particularly susceptible to isoniazid? It may have a very effective autoxidation catalyst or it may be unusually susceptible to the radicals formed. A number of autoxidizable substances are particularly inhibitory to *M. tuberculosis in vitro*, e.g., certain phenolic compounds (36), sulfhydryl compounds, ascorbic acid. Increased oxygen tension, which might increase the autoxidation of intracellular metabolites, also inhibits this organism (37). The catalase of *M. tuberculosis* is more sensitive to isoniazid autoxidation than are other catalases investigated.

(*2*) How does the absence of catalase or peroxidase render *M. tuberculosis* resistant to isoniazid? One or other of these may be the autoxidation catalyst. Neither beef-liver catalase nor horse-radish peroxidase is an autoxidation catalyst for isoniazid, but the mycobacterial enzymes may be different in this respect, as they are in others. A heme compound other than these enzymes, but whose synthesis is related to theirs, may be the catalyst, although the synthesis of other known heme compounds by isoniazid-resistant strains seems to be normal (38). The fact that azide antagonizes isoniazid (39) supports the idea that some heme compound is involved, though it is possible that such a compound located on the bacterial surface serves merely to transport isoniazid into the cell.

(*3*) Why are some hydrazides very much more effective than others against *M. tuberculosis in vitro*? The cross resistance between these hydrazides suggests that they have at

least one step in common in their mode of action. Hydrazides vary considerably in their autoxidation rates (15*c*), and some may be destroyed too rapidly in the external medium; others autoxidized too slowly in the bacilli. Again rates of penetration into the bacilli may vary greatly. Furthermore, it is possible that radicals derived from the hydrazides themselves, such as in the case of isoniazid (*see* 43), are the active radicals.

(*4*) Why does growth inhibition by reduced temperature, streptomycin (40), or lack of a carbon source (41) reduce the bactericidal action of isoniazid? Reduction of the metabolic rate in cells by such conditions protects them against free radicals produced by ionizing radiation. However, the observations of Koch-Weser and co-workers (35), that isoniazid can be recovered quantitatively from cells whose metabolism is reduced by the above conditions, present a more serious problem to the hypothesis, as it is not easy to suggest how autoxidative breakdown could be controlled by metabolism.

(*5*) Holmes and Rubbo (42) have reported that nitrite entered the cells of *M. tuberculosis* but did not antagonize isoniazid. These observations present a problem to an hypothesis of isoniazid action through OH· radical formation, since nitrite destroys this radical. However, the present writer's experience has been that sodium nitrite (200 γ per ml.) does slightly antagonize the action of isoniazid (though it is possible that this is due to slow destruction of isoniazid by the nitrite). Certain other OH· radical-destroying substances, such as phenols (44) and keto-acids, have been reported to antagonize isoniazid.

(*6*) If isoniazid leads to the formation of OH· radicals in *M. tuberculosis*, it would be expected to show some mutagenic effect on this organism, provided that these radicals reach the genetic sites in the cell. Results obtained here on the mutagenicity of isoniazid have so far been inconclusive.

Thus, the examination of these difficulties provides some circumstantial evidence for a free radical hypothesis of isoniazid action, and suggests a number of ways in which the hypothesis may be further tested.

Summary

The specific catalase activities, after rupture of the cells, of BCG, *M. smegmatis*, and *M. phlei* were 2.2 to 3.5, 0.2 to 3.0, and 1.7 L $\times$ gm.$^{-1}$ $\times$ min.$^{-1}$, respectively; while the peroxidase activities (PZ values) were 0.14 to 0.36, 0.034 to 0.20, and 0.17, respectively. Iron, zinc, and cobalt were necessary for maximal catalase and peroxidase levels.

Most of the catalase in *M. smegmatis* and BCG is intracellular. *M. smegmatis* is freely permeable to hydrogen peroxide, while BCG seems relatively impermeable. The peroxidase oxidized polyhydroxyphenols, but not monophenols, guaiacol, aromatic amines, or uric acid.

The catalase and peroxidase were partially inhibited by 10^{-3} M azide, and by heating to 60°C. for ten minutes. Both had optimal activity near pH 7.5, and both were precipitated by three volumes of ethanol. Both activities may belong to the one enzyme.

The degree of inactivation of catalase by isoniazid depended upon its origin, the concentration of isoniazid, the duration and temperature of exposure, the oxygen tension, and the mental ions present. It is suggested that: inactivation results from the metal-catalyzed autoxidation of isoniazid with the formation of free radicals, probably hydroxyl (OH·); inhibition of other enzymes may also be by this mechanism; and inhibition of *M. tuberculosis* is also through free radical formation, a heme compound possibly being the catalyst.

Sumario

La Catalasa y la Peroxidasa en las Micobacterias

Las actividades específicas de la catalasa, después de la rotura de las células, del BCG, del *M. smegmatis* y del *M. phlei* representaron 2.2–3.5, 0.2–3.0 y 1.7 litros $\times$ gramos^{-1} $\times$ minutos^{-1}, respectivamente; mientras que las de la peroxidasa (valores PZ) fueron de 0.14–0.36, 0.34–0.20 y 0.17, respectivamente. Para obtener los valores máximos de catalasa y peroxidasa se necesitaron hierro, cinc y cobalto.

La mayor parte de la catalasa del *M. smegmatis* y del BCG es intracelular. El *M. smegmatis* es ampliamente permeable al peróxido de oxígeno, mientras que el BCG parece relativamente impermeable. La peroxidasa oxidó los polihidroxifenoles, pero no los monofenoles, el guayacol, las aminas aromáticas o el ácido úrico.

La catalasa y la peroxidasa fueron inhibidas parcialmente por la 10^{-3} M azida y por la calefacción a 60°C. durante 10 minutos. Ambas mostraron su actividad óptima a un pH de 7.5 aproximadamente y ambas fueron precipitadas por tres volúmenes de etanol. Ambas actividades pueden pertenecer a una enzima.

La intensidad de la inactivación de la catalasa por la isoniacida dependió de su procedencia, de la concentración de isoniacida, la duración y la temperatura de la exposición, la tensión del oxígeno y los yones metálicos presentes. Se sugiere que: la inactivación proviene de la autooxidación metalocatalizada de la isoniacida con la formación de radicales libres, probablemente hidroxiliones; la inhibición de otras enzimas puede tener lugar también por este mecanismo; y la inhibición del *M. tuberculosis* es igualmente por medio de la formación de radicales libres, siendo posiblemente un compuesto "heme" el catalista.

Resume

Activité catalasique et péroxydasique des mycobactéries

Après rupture des cellules, les activités catalasiques spécifiques du BCG et des *M. smegmatis* et *M. phlei* étaient respectivement de 2,2–3,5, 0,2–3,0 et 1,7 litres × grammes^{-1} × minutes^{-1}; alors que les activités péroxydasiques (valeur PZ) étaient respectivement de 0,14–0,36, 0,34–0,20 et 0,17. Le fer, le zinc et le cobalt étaient nécessaires pour obtenir des niveaux de catalase et de péroxydase maxima.

La majeure partie de l'activité catalasique de *M. smegmatis* et du BCG est intracellulaire. *M. smegmatis* est aisément perméable à l'eau oxygénée, tandis que le BCG est pratiquement imperméable. La péroxydase oxydait les polyhydroxyphénols, mais non les monophénols, le gaïacol, les amines aromatiques et l'acide urique.

La catalase et la péroxydase étaient partiellement inhibées par l'acide 10^{-3} M et par chauffage à 60°C. pendant dix minutes. Toutes deux présentaient une activité optimum au pH 7,5 et étaient précipitées par trois volumes d'alcool éthylique. Chaque enzyme peut posséder les deux activités.

Le degré d'inactivation de la catalase par l'isoniazide dépendait de son origine, de la concentration de l'isoniazide, de la durée et de la température à laquelle se produit cette exposition, de la teneur de l'oxygène et de la présence d'ions-métaux. Il est suggéré que: l'inactivation résulte de l'auto-oxydation due à l'ion catalyseur de l'isoniazide avec formation de radicaux libres, probablement un ion hydroxyl; l'inhibition des autres enzymes peut être aussi due au même mécanisme; l'inhibition du *M. tuberculosis* est aussi due à la formation d'un radical libre, l'hématine agissant probablement comme catalyseur.

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Case Reports

ACUTE ANAPHYLACTOID SHOCK DUE TO BROMSULPHALEIN[1]

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INTRODUCTION

Pyrazinamide, a valuable addition to the chemotherapeutic attack on tuberculosis, has the unfortunate potential of producing serious liver damage (1, 17). Its protracted use consequently requires the serial evaluation of liver function. Perhaps the most widely accepted procedure for this purpose has been the determination of bromsulphalein retention. Although this test has been generally recognized and widely regarded as one of the most valuable of the excretory tests of liver function since its introduction by Rosenthal and White in 1925 (2), its use, particularly in serial fashion, is not totally without danger. Two deaths have recently been attributed to its use: The first, reported by Bjørneboe (3), apparently followed the first injection of bromsulphalein; the second, reported by Walker and Koszalka (4), followed immediately after a second bromsulphalein injection, the patient having shown no reaction following a determination four months previously.

Severe, nonfatal reactions have also been reported sporadically since the first of such reports by Chambers and Moister (5) in 1948. They described a severe anaphylactoid reaction which occurred in an asthmatic female after the first injection of bromsulphalein. The reaction was characterized by asthmatic breathing, decided cyanosis, loss of consciousness, and clonic convulsions. Roth (6), Di Caprio and Troen (7), de Andino and McKeown (8), Soubigou (9), and Caroli (10) also reported similar episodes of severe systemic reactions developing after initial intravenous injections of bromsulphalein.

Morey, Gabuzda, and Scudamore (11) presented observations on three patients who had experienced allergic responses of varying types during studies in which bromsulphalein tests were performed at intervals of one to fourteen or more days. One of these patients had tolerated a number of previous injections without any reaction. Following the inadvertent injection of 0.25 ml. of the dye into the paravascular tissues, however, a local inflammation developed which subsided rapidly on simple treatment, with no apparent sign of any change in the patient's sensitivity. One month later, shortly after an intravenous injection of bromsulphalein, the patient developed both systemic and local reactions which Sievers, Morey, and Samter, in a separate paper (12), characterized as a typical antigen-antibody reaction with release of histamine. Two additional patients had severe pain along the course of the injected vein, with immediate development of urticarial wheals about the venipuncture area; severe inflammation of the entire forearm followed in both cases.

McVay (13) reported a patient who developed a near-fatal reaction following the second of a planned series of bromsulphalein tests after showing no reaction at the time of the original determination three and one-half months earlier. A subsequent skin test with the dye resulted in an immediate, transient, systemic reaction characterized by dyspnea and a slight choking sensation which was followed in the next forty-eight hours by a severe local reaction, including necrosis at the site of injection, swelling of the arm, and epitrochlear and axillary adenopathy. With the development of the latter, the patient also complained of marked malaise and headache and ran a low-grade fever.

Troublesome, but somewhat less serious reactions have likewise been noted by Stempien and Rosenquist (14) who reported local thrombophlebitis following the injection of bromsulphalein in 0.6 per cent of their cases. The writers stressed the fact that bromsulphalein had significant toxic properties when tested in rabbits, and attributed the local reactions to the large volume

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of solution employed in administering 5 mg. per kg.

Taking into consideration the frequency with which bromsulphalein determinations are being made each day, it is somewhat surprising that so few reactions have been reported in the literature. It is probable that the incidence, although low, is still higher than has been reported. It is believed to be worth while, therefore, to add the following two cases to the literature.

Case Reports

Case 1. W. C., a 69-year-old Negro male, was admitted to the hospital on November 20, 1957, for repair of a right inguinal hernia. While in the hospital, a routine chest roentgenogram revealed moderately advanced, active, pulmonary tuberculosis; this was confirmed by microscopy of the sputum on December 28, 1957, and multiple cultures of sputum collected in November and December. Because of an open cavity, the patient was considered eligible for Study XI of the Veterans Administration-Armed Forces protocols, and by random selection was included in a group receiving 300 mg. of isoniazid and 1.5 gm. of pyrazinamide daily. This chemotherapy was started on December 28, 1957.

One of the pre-requisites for selection of patients for Study XI was the determination of bromsulphalein retention, a pretreatment retention of 7 per cent or less being a prime requirement. A routine test was therefore done on December 24, 1957, giving 5 mg. per kg. intravenously. No reaction of any kind was noted. Furthermore, in keeping with protocol requirements, a bromsulphalein test is required at monthly intervals during the use of pyrazinamide. Accordingly, serial tests were done on January 28, 1958, and February 25, 1958; the patient had no reaction at either time. The fourth determination was carried out on March 25, 1958. Although he had not complained at the time, it was subsequently learned that he had had nausea and an episode of emesis shortly after the dye had been administered. He had attributed the episode to "something he ate."

The fifth determination was carried out in routine fashion at 8:30 a.m. on April 29, 1958. The exact length of time for the administration of the dye cannot be definitely stated, but it was given "slowly." Within five minutes after the completion of the injection, the patient complained of a headache, nausea, and had a mild episode of vomiting, but no apparent abdominal pain. Apprehension was marked. He rapidly developed marked pallor and cold clammy perspiration. Radial pulse was imperceptible and blood pressure was unobtainable. In all respects, he presented a typical picture of peripheral vascular collapse, and it was feared that he might die. He did not lose consciousness at any time, but was mentally confused and had great difficulty in speaking. Respirations were labored and musical rales and wheezes were heard anteriorly. Epinephrine, 0.5 ml. (1/1,000), was administered intramuscularly within five minutes of the onset and was the only medication given. His pulse became perceptible within minutes after the injection. The blood pressure could not be detected for fifteen minutes but gradually reappeared and reached normal levels within forty-five minutes. At no time did the patient complain of pain or pruritus. There was no local or generalized urticarial reaction, and the temperature was never elevated. He was kept in bed for the remainder of the day, complaining of marked weakness and malaise during the remainder of the morning, but regained his strength during the afternoon; he was up and about that evening with only mild residual weakness. By the next day, he had completely recovered his previous state of well-being and had an uneventful hospital course thereafter.

Pyrazinamide was continued without interruption until the patient had completed eight months of treatment; PAS was substituted at that time without difficulty. Following the reaction, liver function was evaluated by the serial determination of the serum glutamic oxalacetic transaminase. The patient denied all previous allergic symptoms and gave no family history of allergy. Skin tests with bromsulphalein were contemplated but, because of the fear of possible severe local and systemic reactions following such a procedure, as reported by McVay (13), it was decided not to proceed with the tests. An evaluation of routine blood counts revealed that he had had a leukocyte count of 11,700 on January 28, 1958, with 4 per cent eosinophils; on March 28, 1958, three days following the fourth bromsulphalein determination, the leukocyte count was 8,200 with 14 per cent eosinophils; and on April 1, 1958, the leukocyte count was 9,200 with 6 per cent eosinophils. A differential count on May 28, 1958, revealed that eosinophils had fallen to 1 per cent. Unfortunately, no count had been done at the time of the acute reaction.

Case 2. H. C. McM., a 34-year-old Negro male, was admitted to the hospital on March 4, 1959, with an established diagnosis of pulmonary tuberculosis, moderately advanced, active. On March 5, 1959, a routine bromsulphalein test was carried out by the ward physician in preparation for possible randomization in Study XVIII of the Veterans Administration-Armed Forces protocols (current extension of Study XI). As far as can be determined, it was the patient's first exposure to bromsulphalein. Within five minutes after the intravenous injection of the dye, he complained of generalized itching, vertigo, upper abdominal cramps, and shortly thereafter developed nausea and vomiting. He walked a short distance to the bathroom where he collapsed, remaining unconscious for several minutes. His pulse was rapid and thready; the blood pressure was unobtainable. He was drenched with perspiration and appeared

moribund. Epinephrine, 0.5 ml. administered intramuscularly, was followed by a rapid and dramatic response. Consciousness returned within seconds; blood pressure returned to normal levels within twenty minutes; and all symptoms had disappeared in about forty-five minutes, permitting the withdrawal of sufficient blood to complete the test. The patient denied ever having had any similar attacks previously and gave no history of asthma, hay fever, or other allergic reaction. Due to the severity of the initial reaction, no further attempt was made to repeat additional bromsulphalein tests. Since the initial test revealed a retention of 8.8 per cent, making him ineligible for Study XVIII, the patient was randomized in Study XIX and was assigned to a regimen of isoniazid-PAS.

Comment

As a result of the known tendency of pyrazinamide to produce liver damage, the use of this chemotherapeutic agent in the treatment of tuberculosis requires the concurrent serial evaluation of liver function. Although the determination of bromsulphalein retention has been the standard employed for this purpose and generally meets the criteria of a safe and reliable test, it is apparent that its use may be attended by serious consequences.

Seven reports in the literature have stressed the possibility of a severe reaction at the time of the initial injection of the dye, including one death; three additional reports, including the second death, pointed out the possibility of sensitizing the patient by repeated injections of bromsulphalein at various intervals of time. In the present series, the first patient of this report received three injections of the dye at monthly intervals without any reaction, had mild nausea and emesis following the fourth, and a severe anaphylactoid type of reaction after the fifth. It is believed that this represents a good example of hypersensitivity's developing after repeated intravenous injections of a hapten. At no time was the patient knowingly given the dye intradermally or subcutaneously; however, the possibility of small amounts extravasating into local tissues cannot be entirely excluded. The second patient apparently had an acute anaphylactoid reaction following the initial injection of the dye.

It is not the writers' intent to discredit a well-established and time-tested clinical procedure. Severe reactions are admittedly rare. It is, however, considered advisable to remind all investigators that the repeated use of bromsulphalein in serial fashion must be attended by a keen awareness of its potentiality for producing severe hypersensitivity reactions.

Furthermore, recent studies (15–17) indicate that serial determinations of serum glutamic oxalacetic transaminase offer a reliable and sensitive means of determining early liver damage. Since this test does not require the injection of any dye substance, it is immeasurably safer to use in serial testing. It is believed, therefore, that the transaminase determinations can be safely utilized in the routine evaluation of effective, but potentially hepatotoxic, chemotherapeutic agents in the treatment of tuberculosis.

Summary

Two cases of patients who developed severe reactions to bromsulphalein are described. One patient developed severe shock following the fifth intravenous injection of bromsulphalein, after having had three previous intravenous injections at monthly intervals without any symptoms and only mild nausea and vomiting after the fourth, one month prior to the severe reaction. The second patient developed severe shock with unconsciousness shortly after the initial injection of the dye. Both responded rapidly to intramuscular epinephrine with no apparent permanent residuals.

Survey of the literature reveals that two deaths have been reported following the intravenous injection of bromsulphalein and at least eight cases of similar, severe, nonfatal, anaphylactoid reactions.

The serial determination of serum glutamic oxalacetic transaminase is suggested as a safe substitute in evaluating potential hepatotoxic chemotherapeutic agents in the treatment of tuberculosis.

Sumario

Choque Anafilactoideo Agudo Debido a la Bromsulfaleína

Describense 2 casos de enfermos que manifestaron reacciones graves a la bromsulfaleína. Un enfermo manifestó choque grave a continuación de la quinta inyección intravenosa de bromsulfaleína, después de haber recibido tres inyecciones intravenosas a plazos mensuales sin el menor síntoma y apenas mostrando leves náuseas y vómitos después de la cuarta inyección, un mes antes de la reacción grave. El segundo enfermo manifestó

choque grave con pérdida del conocimiento poco después de la inyección inicial del colorante. Ambos respondieron rápidamente a la epinefrina por vía intramuscular sin aparentes residuos permanentes.

El repaso de la literatura revela que se han comunicado dos muertes consecutivas a la inyección intravenosa de bromsulfaleína y a lo menos ocho casos de semejantes reacciones anafilactoideas graves y no letales.

Se propone la determinación seriada de la transaminasa oxalacética glutámica del suero como substituto inocuo para justipreciar los potenciales agentes quimioterapéuticos hepatotóxicos en el tratamiento de la tuberculosis.

Resume

Choc anaphylactique aigu du a la bromosulphtaleine

Sont décrits 2 cas de malades qui developpèrent des réactions sévères a la bromosulphtaléine. Un malade presenta un choc grave à la suite de la cinquième injection intraveineuse du produit, aprés avoir reçu 3 injections à un intervalle de un mois sans aucun symptome, seulement un léger etat nauséeux et des vomissements a la quatrième injection, un mois avant la sérieuse réaction. Le second malade developpa un choc grave avec perte de conscience peu de temps aprés l'injection initiale de la matière colorante. Tous les deux réagirent rapidement à une injection intramusculaire d'épinephrine avec aucune sequelle apparente.

Il a été jusqu'à présent rapporté d'aprés les statistiques 2 cas de mort, consecutive à l'injection intraveineuse de bromosulphtaléine et au moins 8 cas de reactions anaphylactiques semblables, graves mais non mortelles.

Plusieurs evaluations en série du taux de transaminase serique, glutamique oxalacetique, sont recommendées pour juger d'une façon sure la toxicité pour le foie des agents chimiques utilisés dans le traitement de la tuberculose.

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PERICARDITIS AND PLEURITIS CAUSED BY A RECENTLY DISCOVERED MICROORGANISM, WAKSMANIA ROSEA[1, 2]

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INTRODUCTION

A previously undescribed, mesophilic microorganism was isolated from soil by Lechevalier and Lechevalier in 1957 (1) and named *Waksmania rosea*. In the same year, two other strains with similar morphology were also found in soil. One, a thermophilic strain, was characterized and named *Thermopolyspora biospora* by Henssen (2); and the second, a mesophile, was described and labeled *Microbiospora rosea* by Nonomura and Ohara (3). These new strains were believed to belong to the group of microorganisms lying between the true bacteria and the true fungi and were placed in the order Actinomycetales.

The following case is regarded as representing the first instance of infection in man from which a strain of *Waksmania rosea* was isolated.

P. P. (NYH #773308): A 24-year-old Italian-born white laborer was stabbed in the right chest and back one week prior to admission. A chest roentgenogram taken at that time was reported to be normal. Six days later he developed pleuritic pain over the right lower chest, necessitating hospitalization on July 21, 1957.

Physical examination upon admission revealed a rectal temperature of 38.4°C.; blood pressure 120 systolic, 65 diastolic in mm. of mercury; heart rate, 84 per minute; and respirations, 24 per minute. Positive findings were limited to the chest. Healing stab wounds were present on the right lower chest between the fourth and fifth ribs in the midclavicular line anteriorly, and immediately below the angle of the scapula on the right posteriorly. There was dullness to percussion over the right lower lung field with diminished breath sounds and decreased tactile fremitus. No rales were heard.

Pertinent laboratory studies showed a hemoglobin of 12.5 gm. per 100 ml. and a hematocrit of 34 per cent. The leukocyte count was 12,000 cells per cu. mm. with a differential count of 71 per cent mature polymorphonuclear leukocytes, 11 per cent band forms, and 18 per cent lymphocytes. Values for the serum carbon dioxide combining power, sodium, chloride, potassium, bilirubin, thymol turbidity, cephalin flocculation, transaminase, albumin, globulin, and amylase were all normal. A right lower lobe pneumonia and slight cardiac enlargement were noted on roentgenographic examination of the chest (figure 1). A second-strength tuberculin skin test (purified protein derivative) was strongly positive. Procaine penicillin, 300,000 units, was administered twice daily.

Eight days after admission the patient complained of severe substernal pain which was accentuated during inspiration. A pericardial friction rub was present, and an electrocardiogram showed changes compatible with early pericarditis. No pericardial fluid was detected. There was evidence of a right pleural effusion. Thoracentesis yielded 325 ml. of bloody fluid with a hematocrit of 8 per cent. There were 17,500 leukocytes per cu. mm., the majority of which were polymorphonuclear cells. No microorganisms were seen on Gram or Ziehl-Neelsen stains. Bacterial cultures, including cultures for *Mycobacterium tuberculosis*, were sterile.

The patient's course is depicted in figure 2. His temperature rose to a daily peak of 38.5 to 39.2°C. rectally. The serum transaminase rose to 150 units per ml. per minute (normal 0 to 30 units per ml. per minute). Penicillin was increased to 6 million units daily. The patient was also given 2.0 gm. of streptomycin and 4.0 gm. of erythromycin per day.

Over the next eight days the temperature gradually fell to normal. Evidence of pericarditis, pleural effusion, and pneumonia disappeared. After thirty days of hospitalization the patient was discharged, apparently well, on continued erythromycin therapy, 2.0 gm. per day.

One week after discharge the patient again developed substernal pain. He was again hospitalized on August 31, 1957. On admission the rectal temperature was 37.2°C.; the heart rate was 120 beats per minute; respirations were 28 per minute; and blood pressure 130 systolic, 80 diastolic in mm. of mercury. The patient was in moderate respiratory distress. There were physical signs of a pericardial effusion which included shifting retrosternal dullness and a positive Ewart's sign posteriorly. An apical diastolic gallop was noted.

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FIG. 1. Chest roentgenogram of patient *P. P.* Admission film showing right lower lobe infiltrate and slight cardiomegaly.

Laboratory examination showed a hematocrit of 41 per cent, a leukocyte count of 10,700 per cu. mm. with a differential count of 56 per cent mature polymorphonuclear cells, 3 per cent band forms, 10 per cent eosinophils, 26 per cent lymphocytes, 4 per cent monocytes, and 1 per cent basophils. The erythrocyte sedimentation rate was 45 mm. per hour (Wintrobe).

A pericardiocentesis was performed through an anterior approach. Twenty-five milliliters of serosanguineous fluid were removed. There were 1,400 leukocytes per cu. mm., 86 per cent of which were polymorphonuclear leukocytes. No bacteria were seen. The total protein in the pericardial fluid was 6.4 gm. per 100 ml.

The next day a left pleural friction rub was heard. The temperature rose to 39.4°C. Pericardial and left pleural effusions were noted on a chest roentgenogram (figure 3). On September 3, 1958, 200 ml. of yellow fluid were withdrawn from the left chest through a posterior approach. This had a specific gravity of 1.014. One thousand one hundred and forty leukocytes per cu. mm. were present, the majority of which were polymorphonuclear leukocytes. Total protein was 5.1 gm. per 100 ml.

The diagnosis at this time was recurrent pleuritis and pericarditis on an infectious basis, probably due to a microorganism of low virulence. Tuberculosis was considered a possibility. The patient was digitalized. He was given erythromycin, 3.0 gm. per day; aqueous penicillin, 6.0 million units daily; streptomycin, 2.0 gm. per day; isoniazid, 5 mg. per kg.

Pericardial tamponade developed which required several pericardiocenteses. Fever and pericardial and pleural effusions slowly resolved over the next ten days. All drugs except isoniazid were gradually withdrawn.

A yellowish brown colony without aerial mycelia grew on Sabouraud's agar inoculated with fluid obtained on September 3. Microscopically, the colony was composed of thin, nonbranching, nonfragmenting filaments. No growth occurred on other bacterial media, including beef-heart infusion broth, liver dextrose broth, and thioglycollate. Blood cultures were sterile. The microorganism was thought to be a strain of Nocardia, and a subculture was sent to Dr. C. W. Emmons, Chief Mycologist, U. S. Public Health Service, who deposited a subculture, in turn, in the collection of actinomycetes at Rutgers University.

One month after admission and ten days after discontinuing treatment (except isoniazid), fever, chest pain, and evidence of pericarditis with pleuropericardial effusions recurred. On the assumption that the etiologic agent was a species of Nocardia, penicillin (6.0 million units daily), streptomycin (1.0 gm. per day), and sulfadiazine (3.0 gm. per day) were administered, with rapid improvement in signs and symptoms.

Antimicrobials were continued for two months, during which time the patient appeared well. Routine liver function tests drawn at that time

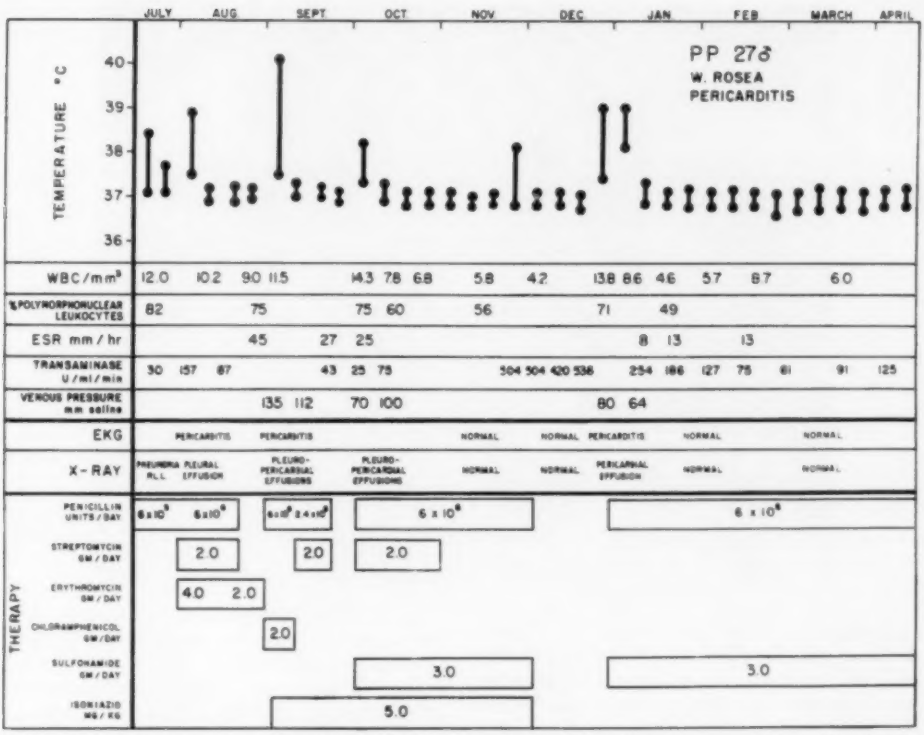

FIG. 2. Nine-month course of patient *P. P.* Each temperature recording represents the maximal and minimal readings during a one-week period.

showed a transaminase of 504 units per ml. per minute, direct bilirubin of 0.4 mg. per 100 ml., alkaline phosphatase of 6.0 Bodansky units. Serum protein electrophoresis, prothrombin time, and the cephalin flocculation test were normal. The liver edge was felt 2 to 3 cm. below the right costal margin and was not tender.

An aspiration biopsy of the liver revealed no significant pathologic abnormalities. It was believed that the hepatic abnormalities might represent drug toxicity. Consequently, all antimicrobials were discontinued. The patient was discharged on the one hundred and twelfth hospital day, at which time liver function tests remained abnormal (*see* figure 2).

One week later, on December 28, 1957, the patient was admitted for the third time with fever, chest pain, and evidence of a pericardial effusion. An antimicrobial regimen of procaine penicillin, 1.2 million units every four hours, and sulfonamide was instituted, with the usual defervescence and disappearance of evidence of pericardial fluid.

Liver function studies demonstrated a transaminase of 254 units per ml. per minute; an alkaline phosphatase of 3.1 Bodansky units; total bilirubin, 1.1 mg. per 100 ml. with 0.4 mg. per 100 ml. in the direct fraction. The erythrocyte sedimentation rate was 8 mm. per hour (Wintrobe).

The patient's subsequent course in the hospital was uneventful. He was discharged after fifty-six days with persistently abnormal liver function studies and moderate left chest pain, which was on occasion pleuritic in nature. Antimicrobials were continued on an outpatient basis for four months. Parenteral and oral penicillin were concomitantly administered so that a concentration of at least 3.2 units per ml. was constantly present in the blood. Sulfadiazine was given in a dosage of 3.0 gm. per day, with maintenance of sulfonamide concentrations in the blood of 6 to 8 mg. per 100 ml.

At present, sixteen months after discontinuing antimicrobials and twenty months after discharge from the hospital, the patient appears entirely well. Physical examination, chest roentgenogram, and electrocardiogram are all normal. Liver function tests show a normal bilirubin and alkaline phosphatase, but the serum transaminase remains elevated at 125 to 330 units per ml. per minute.

FIG. 3. Chest roentgenogram of patient *P. P.*, showing pericardial and left pleural effusion.

Studies of the Microorganism

Studies at the Rutgers Institute of Microbiology and The New York Hospital–Cornell Medical Center have established the following characteristics of this microorganism which has been classified as a strain of *Waksmania rosea*.

Growth characteristics: The strain was subjected to the tests described by Gordon and Mihm (4). On Bennett's agar it formed densely and loosely filamentous colonies that had no aerial (secondary) hyphae at four or eight days. After fourteen days, long, occasionally branching aerial hyphae were present. Spores were formed in pairs at the tip of the long aerial hyphae on very short side-branches and directly on the sides of the aerial hyphae. Spores were not seen on the vegetative (primary) hyphae. The appearance of the vegetative hyphae, aerial hyphae, and spores was identical with that of the type strain of *Waksmania rosea* (figure 4). Sparse aerial hyphae with a few pairs of spores were formed after thirty days on soil extract agar.

The growth of this strain on the various media used by Lechevalier and Lechevalier (1) and its staining properties were very similar to those of the type strain.

As not enough strains of this species were available for study, a distinctive group of correlating, stable, physiologic characteristics cannot be offered for specific identification. Certain properties of this strain, however, served to separate it from strains of *Nocardia asteroides*, the species to which belong most of the aerial hyphae-forming strains of actinomycetes received at Rutgers University from diagnostic laboratories in this country. This strain of *Waksmania rosea* hydrolyzed casein; decomposed tyrosine; and formed acid from arabinose, lactose, maltose, mannitol, alpha-methyl-D-glucoside, raffinose, and xylose. It failed to use propionate as a carbon source. In contrast, strains of *Nocardia asteroides* used propionate as a source of carbon, but were negative in the other reactions given (4, p. 22). Cultures of this strain of *Waksmania rosea* grew at temperatures from 28° C. to 45° C., but not at 50° C.

Animal pathogenicity: A one-week-old culture grown on Sabouraud's agar at 30° C. was emulsified in saline to make a heavy suspension. One-half milliliter was injected intraperitoneally into each of ten 22- to 25-gm. male Swiss albino mice. All mice remained well. Four animals were sacrificed two weeks after inoculation and four more were sacrificed eight weeks after inoculation.

At both the two- and eight-week intervals no abnormalities were noted in the peritoneal

FIG. 4. Aerial hyphae with pairs of spores, type strain of *Waksmania rosea*. (× 1,000)

cavities of the mice. Liver, spleen, and kidneys appeared grossly normal.

Liver, spleen, and kidneys were emulsified together in saline in a Teflon tissue hemogenizer. Five-tenths of a milliliter of the suspension was cultured in duplicate on Sabouraud's agar at 30° C. All cultures remained sterile after thirty days' incubation.

There was thus no evidence under the conditions of this study that this strain of *W. rosea* was pathogenic for mice. Furthermore, there was no indication that the microorganism persisted in mouse tissues for any prolonged period of time.

Drug-susceptibility studies: Sabouraud's agar pour plates containing various concentrations of penicillin, streptomycin, sulfisoxazole, sulfadiazine, chloramphenicol, tetracycline, erythromycin, and novobiocin were heavily seeded with a one-week culture of *W. rosea* grown on Sabouraud's agar at 30° C. Plates were incubated at 30° C. and were read at five and seven days, at which time there was a heavy growth in control plates.

The results of these studies are summarized in table 1. The microorganism appeared to be

TABLE 1

DRUG SUSCEPTIBILITY OF W. ROSEA*

Antimicrobial	Concentration	Growth 0–+++
	U per ml.	
Penicillin	0.05	+++
	0.5	+++
	5.0	+++
	50.0	+++
	mg. per 100 ml.	
Sulfadiazine	3.0	++
	30.0	+
Sulfisoxazole	3.0	+++
	30.0	++
	γ per ml.	
Streptomycin	5	+
	50	0
Tetracycline	1	0
	10	0
Chloramphenicol	1	+++
	10	++
Erythromycin	1	++
	10	+
Novobiocin	10	+
	100	0

* Antimicrobials were incorporated into Sabouraud's agar pour plates. Readings were made at five and seven days.

highly susceptible to tetracycline and moderately susceptible to streptomycin and novobiocin. Slight inhibition was noted with large concentrations of erythromycin and sulfadiazine. Penicillin, chloramphenicol, and sulfisoxazole produced no significant inhibition.

Discussion

It is likely that the patient's recurrent pericarditis and pleuritis represented a smoldering infectious process introduced at the time of the stab wounds. Routine bacterial cultures of the pericardial and pleural effusions were sterile on aerobic and anaerobic media. All cultures for *Mycobacterium tuberculosis* were negative. On a single occasion, culture of fluid obtained from a posterior approach, which may have been either pericardial or pleural in origin, grew a single colony on Sabouraud's agar. Although the microorganism was originally considered to be a species of Nocardia, subsequent studies indicated that it was a strain of the recently described species *Waksmania rosea*.

It has been noted that it is extremely difficult to recover some of the higher bacteria and true fungi in culture. The isolation of a single colony of a potential mycotic pathogen from blood, cerebrospinal fluid, or serous membrane effusions is usually sufficient to establish an etiologic diagnosis if the clinical course is compatible with infection by such an agent (5, 6). It was consequently assumed that the single colony recovered from the serous membrane effusion of this patient was the pathogen responsible for his illness. *Waksmania rosea* has not been noted as a contaminant in this laboratory.

This strain of *W. rosea* was not found to be pathogenic for mice after intraperitoneal inoculation. However, extensive animal investigations were not carried out. No attempts were made to determine pathogenicity for animals other than mice.

Drug-susceptibility studies *in vitro* suggested that tetracycline, novobiocin, and streptomycin were the therapeutic drugs of choice.

It may be noted, however, that during the patient's third hospitalization the pericarditis was treated only with penicillin and sulfonamides. On this regimen, progressive improvement ensued despite the relative nonsusceptibility of the microorganism to these antimicrobials *in vitro*.

Summary

A case is described of pericarditis, pleuritis, and pneumonia believed to have been caused by a recently described microorganism, *W. rosea*. The infection has apparently responded satisfactorily to prolonged antimicrobial therapy. The growth characteristics, animal pathogenicity, and antimicrobial susceptibility of this unusual microorganism are discussed.

Sumario

Pericarditis y Pleuritis Ocasionadas por un Microbio Recién Descubierto, Waksmania Rosea

Describese un caso de pericarditis, pleuritis y neumonía, que parece deberse a un microbio recién descrito, la *W. rosea*. La infección ha respondido al parecer satisfactoriamente a la prolongada terapéutica antimicrobiana. Se discuten las características de proliferación, la patogenicidad para los animales y la susceptibilidad antimicrobiaña de este extraño microbio.

Resume

Pleurite et péricardite causées par un microorganisme récemment decouvert; Waksmania rosea

On décrit un cas de péricardite, de pleurite, et de pneumonie dues croit-on, à un microorganisme récemment décrit—*Waksmania rosea*. Apparemment l'infection répond d'une facon satisfaisante à un traitement antimicrobien prolongé. Les caractéristiques de developpement, la pathogenicité vis a vis de l'animal et la sensibilité aux agents antimicrobiens de ce rare microorganisme sont encore a l'état d'etude.

Acknowledgment

The writers wish to thank Mary P. Lechevalier for examining the strain described here and for supplying the photograph of the type strain of *Waksmania rosea*.

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LOBECTOMY FOR STAPHYLOCOCCAL PNEUMONIA, WITH PNEUMOTHORAX, IN AN INFANT[1]

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Introduction

Staphylococcal pneumonia in infants is not common, but the swiftness with which it strikes and its appalling course compel attention. The high mortality rate is falling. Antimicrobial drugs and aggressive treatment of complications have made the difference.

A very common complication of staphylococcal pneumonia in infants is spontaneous pneumothorax and subsequent empyema. These frightful events are due to rupture of lung abscesses, a characteristic feature of the pneumonia. The clinical course is almost diagnostic: pneumonia in an infant is rapidly progressive over a two- or three-day period, terminating in sudden prostration with extreme difficulty in breathing. Death appears imminent and, in truth, may be.

Pathologically, an intense pulmonary inflammation leads to early suppuration and multiple-abscess formation. The abscesses are subpleural, peripherally located, and are often confined to a reasonably well-localized or well-circumscribed area. They have been described as occurring in clusters. They may be essentially of segmental or lobar distribution. This latter fact can be of vital significance, as will be noted later. The abscesses are prone to rupture, to create a pneumothorax—often a tension pneumothorax. The decision for life or death may be determined by the management of such a pneumothorax.

The treatment of staphylococcal pneumonia is the use of antimicrobial drugs in large doses. A changing variety of antimicrobials has been recommended as resistant strains appear. If, or when, a pneumothorax occurs, it is usually heralded by a sudden and rapid deterioration of the patient's condition. It is now generally recognized that closed drainage of the pleural space is imperative without delay. It is believed that this aggressive concept, in conjunction with the use of antimicrobials, is responsible for the striking improvement in mortality figures in recent years.

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The following case of staphylococcal pneumonia in an infant is presented because it demonstrates how treacherous a complicating spontaneous pneumothorax may be and because a radical departure in therapy was successfully employed.

Case Report

P.S., a five-month-old male, was admitted to Mercy Hospital in the late evening of March 8, 1957. Four days before, he had developed a "cold" with cough, hoarseness, and some fever. Within two days the cough was very severe, and a high fever was present in spite of medication with acetylsalicylic acid. The child continued to nurse and to take his feedings satisfactorily except on the day of admission to the hospital.

Upon hospital admission the temperature was 102°F., the pulse 200 per minute. A loose cough was present, and there was mild inflammation of the pharynx. Respiratory movements were short and rapid, with crying expiration. Suprasternal retraction was present, but no significant costal retraction. Inspiratory crepitant rales were audible, and vocal fremitus was increased. A diagnosis of tracheobronchiolitis was made.

Treatment was instituted as follows: penicillin, 300,000 units daily; chloramphenicol, 100 mg. three times daily; 0.060 gm. of acetylsalicylic acid; and 0.25 per cent neosynephrine nose drops. The temperature the following morning was 104°F. A chest roentgenogram was interpreted as pneumonia of the right lower lobe (figure 1).

On March 10, the child's condition appeared worse. Penicillin was increased, Mistogen[3] was started, and 0.06 mg. of digitoxin was given. A chest roentgenogram (figure 2) divulged a tension pneumothorax on the right. Immediately following bronchoscopy, which revealed only a swollen bronchial mucosa on the right, an intercostal needle was inserted in the right second anterior intercostal space. The needle required reinsertion one hour later to obtain a better position. Regulated continuous chest suction of −12 cm. of water pressure was initiated. The child's breathing improved, and the temperature fell during the next few hours.

On March 11 the condition seemed fairly stable. A Levine tube was inserted for feeding. The chest suction continued to work properly. On March 12 the respirations became more rapid and shallow, with definite episodes of very severe respiratory distress and cyanosis. The temperature rose again. Chest roentgenograms revealed a persistent, al-

FIG. 1. Roentgenogram showing pneumonia of the right lower lobe.

FIG. 2. Roentgenogram showing tension pneumothorax on the right, with shift of the mediastinum to the left. The collapsed lung margin has been retouched.

though varying, pneumothorax, in spite of a normally functioning chest suction apparatus.

During the afternoon of March 12, a right thoracotomy was undertaken under cyclopropane and ether anesthesia. The child was intubated. Multiple, subpleural abscesses of the right lower lobe were found. Several of these small abscesses had ruptured and were leaking air at the time of ex-

ploration. Others were partially or completely sealed over with a filmy fibrin deposit. The abscesses were confined entirely to the lower lobe except for one small unruptured abscess on the under surface of the middle lobe, in the interlobar fissure.

A right lower lobectomy was done without difficulty. The bronchus was closed with silk. The small abscess in the middle lobe was disregarded. It was thought inadvisable to prolong the procedure to the extent that a supplemental middle lobe lobectomy would require. Two intercostal catheters were inserted for postoperative suction, and the chest wall was closed in the usual fashion. Colonies of *Staphylococcus aureus* and *albus* were later cultured from the surgical specimen.

The postoperative course was uneventful. Heavy doses of penicillin alternating with penicillin–streptomycin were given every six hours. The upper and middle lobes expanded quite readily to fill the pleural space. The intercostal catheters were removed on the third postoperative day. All medication was stopped on March 23, and the patient was dismissed from the hospital on March 26, fourteen days after thoracotomy and eighteen days following hospital admission.

Follow-up examination six months later revealed a normally developed child. Lung sounds on the right were normal. A chest roentgenogram disclosed a well-expanded lung on the right, with nearly normal volume. There was some evidence of an enlarged thymus.

Discussion

Experience with spontaneous pneumothorax, of whatever etiology, has taught that thoracotomy is indicated if the pneumothorax cannot be controlled by closed drainage and suction. This principle, however, has apparently seldom been applied to the pneumothorax associated with rupture of lung abscesses in staphylococcal pneumonia in infants. Perhaps its application is seldom necessary. Nonetheless, the mortality still associated with this disease and complication is undoubtedly due, at least in part, to the insult of a pneumothorax that is not controlled or controllable by the usual means of closed drainage.

Resection has been indicted in the medical literature as entailing a sacrifice of useful lung tissue. Although this charge loses its import in the presence of a dangerously degenerating clinical situation, the possible lobar or segmental distribution of the abscesses does presage an economical resection and, far more fundamental, a favorable outcome. Resection is certainly not indicated if simpler measures succeed but, conversely, it may represent the only hope if simpler measures fail.

Summary

A case of staphylococcal pneumonia in an infant is presented in which prompt and customary management of a complicating spontaneous pneumothorax was inadequate, and thoracotomy with resection was required.

The infant was dismissed from the hospital, cured, fourteen days after resection of the right lower lobe. Thoracotomy and resection are indicated if the pneumothorax commonly complicating this pneumonia cannot be controlled by closed drainage and suction.

Sumario

La Lobectomía por Neumonía Estafilocócica, con Neumotórax, en un Lactante

Preséntase un caso de neumonía estafilocócica en un lactante, en el cual resultó inadecuada la atención rápida y habitual prestada a un neumotórax espontáneo que complicó el caso, y se requirió la toracotomía con resección.

El lactante fué dado de alta, curado, a los catorce días de la resección del lóbulo inferior derecho. Hállanse indicadas la toracotomía y la resección si no cabe cohibir con el drenaje cerrado y la succión el neumotórax que complica frecuentemente dicha neumonía.

Resume

Lobectomie pour pneumonie à staphylocoques avec pneumothorax chez un nourrisson

L'auteur présente un cas de pneumonie à staphylocoques chez un nourrisson, dans lequel le traitement ordinaire et immédiat d'un pneumothorax spontané s'est montré insuffisant et où une thoracotomie avec résection fut nécessaire.

L'enfant sortit de l'hôpital, guéri, quatorze jours après la résection du lobe pulmonaire inférieur droit. La thoracotomie avec résection est indiquée si le pneumothorax qui complique souvent ce type de pneumonie ne cède pas au drainage en circuit fermé et à l'aspiration.

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Editorial

The Importance of Postoperative Drug Therapy for Pulmonary Tuberculosis

The phthisiologist is often asked to recommend a course of antituberculous chemotherapy following pulmonary resection for tuberculosis. Considering the vast experience which has been accumulated since the discovery of streptomycin, it might be supposed that this could be done with little difficulty. Actually, little coordinated information on the subject is available. Indeed, it appears that decisions often have little scientific foundation, depending upon the nebulous entity commonly known as "judgment." Yet, it would be inaccurate to say that little is known. A review of the literature and one's own experience yields a considerable amount of information which, although apparently conflicting, may be correlated to yield a fairly accurate understanding of the problem.

One of the reasons for conflicting reports and opinions is the use of various drug regimens, necessitated by the introduction of new drugs and the evolution of knowledge regarding the mode of action of drugs in use. A review of the early experience of many surgeons[1] indicates that pulmonary resection accompanied by short courses of antituberculous chemotherapy yielded approximately 70 to 80 per cent good results compared with less than 40 to 50 per cent without drug therapy. As more information accumulated regarding the use of antituberculous drugs, surgical results continued to improve. At present, long-term therapy with combinations of streptomycin, para-aminosalicylic acid (PAS), and isoniazid of eighteen to twenty-four months' duration is frequently prescribed whether or not surgery is performed.[2]

It is evident that various drug regimens have different potentialities. Although some clinical experiences[3] fail to indicate a difference between the results of daily and twice-weekly streptomycin combined with daily PAS, the British[4] report a lower incidence of resistant organisms under the former regimen. Isoniazid daily administered along with streptomycin or PAS daily appears superior to daily streptomycin combined with PAS.[5] Isoniazid alone is claimed to be as efficacious as isoniazid with PAS in the treatment of noncavitary tuberculosis.[6]

In view of these considerations, it might be expected that the relatively high post-resection relapse rates which occurred when streptomycin and PAS were used would not be duplicated in patients receiving isoniazid regimens.[7] Bell and associates[8] reported an 8.4 per cent relapse rate with streptomycin–PAS following resection for cavitary pulmonary tuberculosis for patients whose expectorations did not contain culturable tubercle bacilli. Viable tubercle bacilli were found in the tissues removed at 38 per cent of the operations. Holland and associates[9] reported from the same institution a complication rate of 10 per cent following resection for cavitary disease in patients whose expectorations had contained drug-susceptible tubercle bacilli within three months prior to surgery. Data concerning cultures from tissues were not included, but it may be assumed that at least some of these patients might have attained a noninfectious state within the period of three months prior to surgery. The similar results reported by Bell and Holland may be explicable on the basis that clinical criteria rather than criteria based upon examination of the excised tissue were employed, and as a result both groups included noninfectious as well as infectious patients. Because of this lack of homogeneity, such surgical reports cannot be employed as a basis for comparison of drug regimens.

It seemed advisable to the writer, therefore, to review his own experience, dividing the patients into infectious and noninfectious groups on the basis of results of culture of both expectorations and surgically removed tissue. Between January

[1] Kelley, W. O., and Pecora, D. V.: J. Thoracic Surg., 1952, *24*, 510.

[2] American Trudeau Society: Am. Rev. Tuberc., 1958, *78*, 656.

[3] Bachrach, C. A.: Tr. Seventeenth Conference on Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1958, p. 105.

[4] Report by the Laboratory Subcommittee of the Tuberculosis Chemotherapy Trials Committee, Medical Research Council: Lancet, 1953, *265*, 217.

[5] Livings, D. G.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 20.

[6] Phillips, S.: Tr. Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1959, p. 29.

[7] Mitchell, R. S.: Am. Rev. Tuberc., 1957, *76*, 508.

[8] Bell, J. W., *et al.*: Am. Rev. Tuberc., 1957, *75*, 538.

[9] Holland, R. H., *et al.*: J. Thoracic Surg., 1956, *31*, 83.

1, 1950, and January 1, 1957, we performed pulmonary resections upon 45 patients receiving multiple-drug therapy who harbored drug-susceptible tubercle bacilli at the time of surgery. There was a tuberculous complication rate of 28 per cent in the group which received streptomycin–PAS and 13 per cent in the group which received different isoniazid regimens. Part of the difference in results is explicable on the basis that half of the complications in the streptomycin–PAS group occurred in patients whose therapy had been interrupted. During the same period, 265 noninfectious patients receiving multiple-drug therapy were operated upon. There was a tuberculous complication rate of 2.6 per cent in the streptomycin–PAS group and 0.9 per cent in the group which received the isoniazid regimens. Obviously, these results cannot be regarded as conclusive, but they are not incompatible with the evidence already reviewed that isoniazid combinations yield superior results to streptomycin–PAS.

In comparing surgical results, other factors must be considered. The duration of therapy is of the utmost importance and, unfortunately, is rarely reviewed in detail. In our series, patients were given postoperative drug therapy for various periods, occasionally in excess of ten months. After eliminating patients who received intermittent therapy, it was found that, in the infectious group, there was a tuberculous complication rate of 14 per cent in those who received the drugs for six months or less and of 15 per cent in those who received therapy for more than 6 months. In the noninfectious group, 3 per cent of the tuberculous complications occurred in those who received the drugs for six months or less, and no complications in the patients who received longer therapy. These results appear to agree with those of Bell and associates,[7] who reported that postoperative chemotherapy appeared unrelated to the incidence of relapse if it was maintained for a minimum of six months. Since there is little information available regarding the results of postoperative chemotherapy of longer duration, perhaps several years, it appears possible that they might be improved under the latter conditions.

Another factor which must be considered in assessing the effects of any therapy is the amount of disease. This has been demonstrated for nonsurgical cases by Livings[8] and by the writer[10] for pulmonary resection. Adequate and prolonged antituberculous chemotherapy following pulmonary resection is more important for those patients who have a great deal of residual disease than for those who do not.[11]

[10] Pecora, D. V., and Yegian, D.: Am. Rev. Tuberc., 1957, *75*, 781.

[11] Pecora, D. V.: J. Thoracic Surg., 1958, *36*, 190.

Certain surgical factors influence the results of antimicrobial therapy to a considerable extent. It is the goal of the surgeon to remove cavitary disease, since it has long been apparent that obliteration of cavities is often followed by clinical cure of pulmonary tuberculosis. However, one must be aware of the possibility that, after thoracotomy, a pleural space filled with air or fluid may exist and that such spaces may have the same potentialities for infection as pulmonary cavities. If the patient is noninfectious at the time of surgery, few of these pleural spaces become infected. If the patient harbors culturable tubercle bacilli at the time of surgery, however, there is considerable danger that empyema will occur.[12] Usually antituberculous chemotherapy with combinations of streptomycin, PAS, and isoniazid will prevent the occurrence of empyema, at least during the duration of chemotherapy.[13]

The patients in our series, reviewed in the preceding paragraphs, had comparable surgery and residual disease.

It should be noted that not all surgeons agree that the state of infectiousness of the patient at the time of pulmonary resection is important. Overholt[14] and Wilson[15] have stated that preoperative control of infectiousness is of little prognostic importance provided the patient is operated upon early during the first course of antituberculous therapy and provided that certain criteria are fulfilled. The criteria include administration of drugs for a sufficient time to eliminate toxic symptoms and excessive sputum, exclusion of patients without adequate pulmonary reserve, and resection of the only threatening pulmonary lesion. They reported only one "fistula" in 231 resections. These results are certainly superior to those of most reported series, so much so in fact as to lead to the suspicion that the element of selection is a most important determining factor. One is reminded of the experience of Chamberlain[16] who was able by careful selection to report similar surgical results with and without drug therapy.

Since clinical experiences are no more than suggestive, it would appear profitable to review pertinent laboratory data on the subject of drug therapy. Assuming that the surgeon succeeds in removing all cavitary disease and in eliminating pleural spaces, the results of postoperative antituberculous therapy depend upon the behavior of

[12] Pecora, D. V.: J. Thoracic Surg., 1956, *32*, 216.

[13] Pecora, D. V.: Dis. Chest, 1959, *36*, 31.

[14] Overholt, R. H.: Discussion, J. Thoracic Surg., 1955, *29*, 160.

[15] Wilson, N. J.: Discussion, J. Thoracic Surg., 1956, *31*, 91.

[16] Chamberlain, M.: Discussion, J. Thoracic Surg., 1953, *26*, 485.

the residual disease. In attempting to correlate tissue bacteriology with type and duration of antituberculous therapy, we[17] have found that the proportion of specimens yielding viable tubercle bacilli decrease to a minimum of approximately 10 per cent at the end of eight to ten months of therapy. McDermott[18] has submitted convincing evidence that, even when culturable bacilli are not recovered from the tissues, hidden or "latent" infection may be present which can later become active. He also presents a considerable amount of evidence that during prolonged antimicrobial therapy drug-susceptible organisms may remain in the tissues as "persisters." Whether or not indefinitely prolonged antimicrobial therapy would improve these results is not known. One might speculate that host immunity could succeed in eliminating the small number of bacterial "persisters" if given sufficient time. Yegian and Budd[19] reported disappearance of evidence of infection and reversal of the tuberculin skin test in guinea pigs followed for three and one-half years after infection with small numbers of virulent tubercle bacilli. It is Auerbach's[20] opinion that drugs merely speed up the healing process of a tuberculous infection and that recovery of tubercle bacilli from necrotic tuberculous foci is related to the stage of anatomic healing. Canetti[21] found that the rate of recovery of viable tubercle bacilli from caseous pulmonary lesions decreased with diminution in signs of activity.

Data from different sources reveal that there is no evidence that any type of antituberculous chemotherapy can ensure complete elimination of tuberculous infection. Moreover, since tuberculosis is a generalized infection, surgery can do no more than remove threatening foci. Therefore, it must be concluded that the immunologic mechanisms of the host influence to a great extent the results of both drug therapy and surgery. Yet, these considerations should in no way minimize the importance of either of the latter.

Faced with the necessity of formulating a schedule for post-resection antimicrobial therapy, the clinician is fortunate in having available the operative findings and surgical specimen. Usually he can be reasonably certain that the patient has no remaining cavitary disease or, if a pleural space exists, that it can be surgically eliminated in most instances. Although the methods usually employed in culturing the surgically removed tissues are relatively crude and subject to sampling errors, the results correlate so well with the subsequent clinical course of the patient as to indicate that they may be relied upon for the purpose of prognostication and treatment.

At present it appears preferable to prescribe two antituberculous drugs following pulmonary resection, although there is some evidence that isoniazid alone may be adequate to control noncavitary disease. Isoniazid combined with daily streptomycin or PAS can be expected to yield better results than streptomycin–PAS. It would appear wise to consider patients who harbor culturable tubercle bacilli at the time of surgery separately from those who do not. As data obtained by culture of resected tissue indicate that at least eight to ten months of combined antituberculous therapy is necessary for maximal suppression of tubercle bacilli, it would appear desirable to treat all patients for a minimum of a year for good measure. Future experience may indicate that therapy of longer duration will yield better results. Patients who harbor culturable organisms at the time of surgery should probably receive a full year of therapy postoperatively; those who do not should receive sufficient therapy postoperatively to complete a full year of therapy. In all probability, patients who have a large amount of residual disease should be treated longer.

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[17] PECORA, D. V.: Am. Rev. Tuberc., 1959, *79*, 41.

[18] MCDERMOTT, W.: Yale J. Biol. & Med., 1958, *30*, 257.

[19] YEGIAN, D., AND BUDD, V.: Am. Rev. Tuberc., 1955, *72*, 539.

[20] AUERBACH, O., *et al.*: Am. Rev. Tuberc., 1957, *76*, 987.

[21] CANETTI, G.: Bull. union internat. tuberc., 1954, *24*, 148.

Notes

THE EFFECT OF HISTOPLASMIN IN VITRO ON THE MIGRATION OF LEUKOCYTES FROM GUINEA PIGS EXPERIMENTALLY INFECTED WITH HISTOPLASMA CAPSULATUM[1]

In 1928, Rich and Lewis[2] found that the migration of leukocytes and of splenic cells from tuberculous guinea pigs was inhibited, *in vitro*, by concentrations of tuberculin that had no effect on the migration of these cells from normal animals. This finding was confirmed, first by Aronson,[3] and later by Heilman, Feldman, and Mann.[4] Since then, Moen[5] demonstrated the specific *in vitro* sensitivity of splenic cells from guinea pigs infected with group C streptococci to an extract of these organisms; and Heilman, Howard, and Carpenter[6] showed that the migration of splenic cells from *Brucella suis*-infected guinea pigs was inhibited by suspensions of heat-killed *Brucella suis*. These studies have all been concerned with bacterial infections.

The present study was undertaken to determine whether or not the leukocytes from guinea pigs with mycotic infections, in which a delayed type of hypersensitivity is known to occur, would also develop a leukocytic hypersensitivity. The infection selected for study was histoplasmosis.

Experimental approach: A group of 24 guinea pigs that were negative cutaneous reactors to both histoplasmin and brucellergen was divided into two groups of 12 each. One group was infected with *Histoplasma capsulatum*; the other served as uninfected controls. At three- and four-day intervals following infection, leukocyte migration studies and skin tests were performed on 2 animals from each group over a ten-week period.

Infection of guinea pigs: Five-tenths of a milliliter of a suspension, in Ringer's solution, of about 10,000 living yeast-phase cells of the Duke strain of *H. capsulatum* were injected intraperitoneally.

Skin tests: One-tenth of a milliliter of a 1:100 dilution of histoplasmin (Parke Davis, Bio 498), and 0.1 ml. of a 1:10,000 dilution of brucellergen (Sharpe and Dohme, H4108) were injected intradermally into separate sites. The injection sites were examined two hours after injection, and again at twenty-four and forty-eight hours. A histoplasmin skin test was considered positive if an area of induration 5 × 5 mm. or greater appeared in twenty-four hours or more, and a brucellergen skin test was considered positive if an area of erythema, edema, and induration of 2 cm. in diameter appeared in twenty-four hours or more.

Leukocyte migration studies: The method of performing the leukocyte migration studies was essentially the same as that previously used in this laboratory,[7, 8] except that the blood (4 ml.) was drawn aseptically by cardiac puncture into a syringe wetted with a 1:2,500 dilution of heparin. The heparin replaced the Amberlite® decalcifying agent and eliminated the necessity of using Tyrode's solution. The blood was centrifuged in the cold, and the plasma was removed and kept on ice while the tissue cultures were being prepared. The buffy coats were clotted by placing the tubes with the sedimented cells in the 37°C. incubator from fifteen to thirty minutes. These clotted buffy coats were later cut into small fragments (about 1 mm. square) and washed in cold Ringer's solution.

Dilutions of histoplasmin, control broth (uninoculated broth used to prepare histoplasmin), and brucellergen were prepared in the plasma. Four fragments of buffy coat were arranged in a square on a coverglass with a drop of a plasma reagent mixture which, when clotted, served as a tissue culture for the migration studies.

Cytotoxic indices for the histoplasmin were determined by dividing the average migration distance of leukocytes in a dilution of histoplasmin by the average migration distance of leukocytes in the same dilution of control broth. The cytotoxic indices for the brucellergen were obtained by dividing the average migration distance of leukocytes in the dilution of brucellergen by the average migration distance of leukocytes in plasma alone. Plasma, rather than broth, was used with brucellergen since brucellergen is not the filtrate of a broth culture, but rather the protein nucleate extract of Brucella cells.

On the basis of a preliminary study with the leukocytes from normal guinea pigs, dilutions of 1:1,000, 1:5,000, and 1:10,000 of histoplasmin and

[1] This study was supported by grant No. E-882 (C2R1) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service; and by a grant from the Kentucky Heart Association.

[2] Rich, A. R., and Lewis, M. R.: Proc. Soc. Exper. Biol. & Med., 1927–1928, *25*, 596.

[3] Aronson, J. D.: J. Exper. Med., 1931, *54*, 387.

[4] Heilman, D. H., *et al.*: Am. Rev. Tuberc., 1944, *50*, 344.

[5] Moen, J. K.: J. Exper. Med., 1936, *64*, 355.

[6] Heilman, D. H., *et al.*: J. Exper. Med., 1958, *107*, 319.

[7] Scherago, M., *et al.*: Ann. Allergy, 1957, *15*, 1.

[8] Hall, H. E., and Scherago, M.: Am. Rev. Tuberc., 1957, *75*, 807.

control broth, and a 1:10,000 dilution of brucellergen were selected for this study. A 1:1,000 dilution of histoplasmin and a 1:10,000 dilution of brucellergen were found to be the highest concentrations that did not markedly inhibit the migration of leukocytes from normal guinea pigs *in vitro*. The 1:5,000 and 1:10,000 dilutions of histoplasmin were used along with the 1:1,000 dilution to determine whether higher dilutions would inhibit the migration of the leukocytes from the infected guinea pigs.

Analyses of the results of the migration tests obtained with the leukocytes of the control animals over the ten-week period are shown in table 1. With the 1:1,000 dilution of histoplasmin, the range of cytotoxic indices was from 1.24 to 0.76, with a mean of 0.98 and a standard deviation of 0.13; with the 1:5,000 dilution the range of cytotoxic indices was 1.20 to 0.65, with a mean of 0.98 and a standard deviation of 0.14; with the 1:10,000 dilution the range of cytotoxic indices was 1.28 to 0.72, with a mean of 0.98 and a standard deviation of 0.14. With the 1:10,000 dilution of brucellergen, the range of cytotoxic indices was 1.24 to 0.52, with a mean of 0.84 and a standard deviation of 0.13.

If a cytotoxic index that is less than the mean cytotoxic index of the normal leukocytes minus twice the standard deviation is indicative of significant sensitivity,[9] then with the 1:1,000 dilution of histoplasmin a cytotoxic index below 0.72 would be indicative of significant sensitivity. With both the 1:5,000 and 1:10,000 dilutions, a cytotoxic index below 0.70 would be indicative of significant sensitivity. With the 1:10,000 dilution of brucellergen, a cytotoxic index below 0.58 would be indicative of significant sensitivity.

With the infected animals, the skin test reactions (to a 1:100 dilution of histoplasmin) and the cytotoxic indices obtained, using the 1:1,000 dilution of histoplasmin, are shown in table 2. The mean cytotoxic index for the 3 animals tested the first week after infection was 1.04, and the skin tests were all negative. During the second week, the mean cytotoxic index of the 4 animals tested dropped sharply to 0.78, with the leukocytes from 2 of the animals (*7* and *8*) showing cytotoxic indices within the range of significant inhibition (0.71 and 0.63, respectively). Neither of these animals were positive cutaneous reactors to histoplasmin at this time, but one of the other animals (*9*) tested along with numbers *7* and *8* exhibited a doubtful cutaneous reaction although its cytotoxic index (0.93) remained well above that of significant inhibition. The third week, only 3 of the 4 animals were skin tested (*11*, *2*, and *3*) and they were all positive. The fourth guinea pig (*12*) died shortly after it was bled for the leukocyte migration test. The cytotoxic indices of none of these 4 animals fell within the range of significant inhibition, and the mean cytotoxic index for the 4 was 0.88. In the fourth week, however, all of the animals tested (*4*, *6*, *8*, and *9*) showed cytotoxic indices of 0.58 or below, with a mean of 0.54, indicating significant sensitivity to the histoplasmin. All of the animals were also positive cutaneous reactors. From this point on, all of the infected animals tested up to the termination of the study after ten weeks were positive cutaneous reactors to histoplasmin. Throughout the remainder of the study, the cytotoxic indices of all of the animals tested remained below 0.72.

At no time was a cytotoxic index of 0.72 or below obtained with a noninfected control animal with the 1:1,000 dilution of histoplasmin. With the 1:5,000 dilution of histoplasmin, during only one week was a mean cytotoxic index below 0.70 obtained with the leukocytes from the infected guinea pigs, and in that week only 2 animals were tested, with cytotoxic indices of 0.80 and 0.51. Of the twenty-one samples of leukocytes that were examined for migration with this dilution, only 6 had cytotoxic indices indicative of significant inhibition. With the leukocytes from the control animals, only one cytotoxic index occurred that was below 0.70.

With the 1:10,000 dilution of histoplasmin, not once during the ten weeks was a mean cytotoxic index below 0.70 obtained with the leukocytes from the infected animals. Of the 34 samples of

TABLE 1

ANALYSES OF THE RESULTS OF THE MIGRATION TESTS WITH THE LEUKOCYTES OF THE CONTROL GUINEA PIGS

Dilution of Reagent	Number of Tests	Range of Cytotoxic Indices	Mean	Standard Deviation	Mean Minus Twice the Standard Deviation
Histoplasmin 1:1,000	34	1.24–0.76	0.98	0.13	0.72
Histoplasmin 1:5,000	19	1.20–0.65	0.98	0.14	0.70
Histoplasmin 1:10,000	33	1.28–0.72	0.98	0.14	0.70
Brucellergen 1:10,000	33	1.24–0.52	0.84	0.13	0.58

TABLE 2

CYTOTOXIC INDICES WITH 1:1,000 HISTOPLASMIN, AND SKIN TEST REACTIONS OF INFECTED GUINEA PIGS TO 1:100 HISTOPLASMIN

Week after Infection															
1			2			3			4			5			
GP*	ST†	CI‡	GP	ST	CI	GP	ST	CI	GP	ST	CI	GP	ST	CI	
3	−	1.00	7	−	0.71	11	+	0.76	4	+	0.54	10	+	0.56	
4	−	1.15	8	−	0.63	12	0	0.83	6	+	0.52	11	+	0	
6	−	1.28	9	±	0.93	2	+	0.96	8	+	0.58	2	+	0.66	
			10	−	0.86	3	+	0.95	9	+	0.51	3	+	0.57	
Mean		1.04	Mean		0.78	Mean		0.88	Mean		0.54	Mean		0.60	
6			7			8			9			10			
GP	ST	CI	GP	ST	CI	GP	ST	CI	GP	ST	CI	GP	ST	CI	
4	0	0.71	9	+	0.48	4	0	0.60	8	+	0.66	10	+	0.41	
			10	+	0.58	3	+	0.56	9	+	0.62	11	+	0.61	
7	+	0.56	11	+	0	6	+	0.65				2	+	0.63	
8	0	0.61	2	+	0.71	7	+	0.56				4	+	0.63	
Mean		0.63	Mean		0.59	Mean		0.59	Mean		0.64	Mean		0.56	

Cytotoxic indices below 0.72 indicate significant sensitivity to histoplasmin.
* GP = guinea pig number
† ST = skin sensitivity; + = positive − = negative ± = doubtful 0 = not done
‡ CI = cytotoxic index

leukocytes examined for migration, only 4 had cytotoxic indices below 0.70. No cytotoxic index below 0.70 was obtained with the leukocytes from the control animals.

With the 1:10,000 dilution of brucellergen, the specificity control, the mean cytotoxic index for the infected animals fell no lower than 0.77 (the seventh week), and at no time did a cytotoxic index fall below 0.58 (indicative of significant sensitivity to brucellergen). With the uninfected animals only one cytotoxic index below 0.58 was obtained.

Periodic skin tests with histoplasmin and brucellergen on the control animals were always negative. At no time did the infected animals exhibit skin sensitivity to brucellergen.

* * *

The results of this study indicate that the leukocytes from guinea pigs infected with *H. capsulatum* are significantly more sensitive to a 1:1,000 dilution of histoplasmin than are the leukocytes from normal guinea pigs. This finding is in accord with the results of other workers[3, 5, 6] who have demonstrated cellular sensitivity of infected animals to the products of the infecting organism.

The inconsistent results obtained with the 1:5,000 dilution of histoplasmin with the leukocytes from the infected animals, and to a lesser extent with the 1:10,000 dilution, emphasize the necessity of selecting, on the basis of preliminary experiments, a concentration of histoplasmin strong enough to inhibit migration of leukocytes from infected animals without inhibiting the migration of the leukocytes from normal animals.

That the sensitivity in the presence of histoplasmin is specific and not the result of a weakening of the leukocytes by the infection is supported by the reaction of the leukocytes from the infected animals to brucellergen, the extract of an organism entirely unrelated to *H. capsulatum*. The leukocytes from the infected animals migrated in the presence of the brucellergen to the same degree as did the leukocytes from normal animals. However, the exact degree of specificity of the sensitivity remains to be determined. Products from other organisms closely related to *H. capsulatum* (coccidioidin and blastomycin, the filtrates of broth cultures of *Coccidioides immitis* and *Blastomyces dermatitidis*, respectively) have been shown to give positive skin reactions when injected into subjects with histoplasmosis. Whether or not this cross reactivity occurs at the

level of leukocytic sensitivity also is a point that needs to be determined.

Now that the leukocytes in experimental histoplasmosis have been shown to be specifically sensitive to histoplasmin, it is possible that the leukocytes in human and canine histoplasmosis are similarly hypersensitive. That human leukocytes do become hypersensitive has been demonstrated by Hall and Scherago[8] in their study of human tuberculosis.

In summary: The leukocytes of guinea pigs experimentally infected with about 10,000 living yeast-phase cells of *Histoplasma capsulatum* have been found to develop significant specific sensitivity, *in vitro*, to a 1:1,000 dilution of histoplasmin.

Skin sensitivity first appeared in the infected animals during the third week after infection, while inhibition of leukocyte migration appeared as early as the second week in 2 of 4 animals tested. After the fourth week the migration of leukocytes from all of the infected animals was inhibited. Both skin and leukocytic sensitivity persisted through the ten-week period of the study. Consistent significant sensitivity was not found with the 1:5,000 or 1:10,000 dilutions of histoplasmin. The sensitivity was considered specific since brucellergen had no significant inhibitory effect on the migration of the leukocytes from the infected animals.

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THE EFFECT OF CHEMOTHERAPY ON THE TUBERCULIN SENSITIVITY OF LEUKOCYTES FROM TUBERCULOUS PATIENTS[1]

In their studies of leukocyte sensitivity to tuberculin, Hall and Scherago found that the sensitivity of the leukocytes of guinea pigs experimentally infected with *Mycobacterium tuberculosis* H37Rv increased with the progression of the disease,[2] and that this sensitivity decreased after treatment with streptomycin sulfate and isoniazid.[3] These findings suggested a similar study on the sensitivity of leukocytes of patients with active tuberculosis.

Experimental approach: Eight male patients, twenty-five to thirty-eight years old, 5 of whom had moderately advanced, and 3, minimally advanced tuberculosis, were selected for the experiment. All of them had had positive skin reactions to the intracutaneous injection of PPD and had not been previously treated for tuberculosis. They were free from such complicating conditions as coronary disease, diabetes, cancer, et cetera. Sputum smears and cultures taken from the 8 patients on admission were positive for acid-fast bacilli, and all of the cultures had positive reactions to catalase[4] and to peroxidase.[5]

The leukocytes were tested for hypersensitivity on the day of admission and at six-week intervals thereafter, corresponding to the periodic medical examinations. At each examination period, smears of the concentrates of three twenty-four-hour sputum specimens were examined. When acid-fast bacilli were not observed in the smears, the concentrates were cultured for *M. tuberculosis*.

The duration of the testing period was sixty weeks for 3 of the patients. The other 5 patients left the hospital before this period; 2 after thirty-six weeks, 2 after forty-two weeks, and one after fifty-four weeks.

Chemotherapy: All of the patients were started on chemotherapy upon confirmation of the diagnosis, and continued to receive it throughout the course of the study. Routinely this was isoniazid and dihydrostreptomycin, but in some cases para-aminosalicylic acid and viomycin were also used.

Testing for leukocytic sensitivity: The method used for testing for leukocytic sensitivity was the migration technique of Rich and Lewis as modified in this laboratory.[6, 7] After the migration distances were determined in the presence of glycerol broth and of Old Tuberculin (OT), the degree of inhibition of migration of the leukocytes for each patient was expressed as a ratio, the cytotoxic index, obtained by dividing the migration figure derived in the presence of OT by that derived in the presence of glycerol broth. The mean cytotoxic index for all of the patients was then determined. The results are shown in table 1.

As may be seen from table 1, the cytotoxic indices on admission ranged from 0.53 (*Patient 548*) to 0.74 (*Patient 702*) with a mean cytotoxic index of 0.63. The leukocytes from all of the patients began to show a decrease in sensitivity by the eighteenth week. By this time, also, the sputum from 7 of the 8 patients had become negative for acid-fast bacilli. The time when the sputum became negative is shown in the table by an asterisk at the cytotoxic index. The sputum of the eighth patient did not become negative until the thirtieth week. From the eighteenth week on there was, in general, a progressive decrease in leukocytic sensitivity, as indicated by the mean cytotoxic indices. There was a marked increase in the cytotoxic index of each patient from the day of admission, before chemotherapy was initiated, until the last day on which the leukocytes were tested for sensitivity. This increase was also reflected in the mean cytotoxic indices for the 8 patients, from 0.63 before treatment to 0.86 during the thirty-sixth week, the last time the test was done on all 8 patients.

The cytotoxic indices at the end of thirty-six weeks of therapy ranged from 0.80 (*Patient 573*) to 0.93 (*Patient 615*), with a mean cytotoxic index of 0.86. By the forty-second week of testing, *Patients 615* and *702* had left the hospital and were no longer available for testing. By the end of this period the cytotoxic indices for the remaining 6 patients ranged from 0.80 (*Patient 548*) to 0.94 (*Patient 551*), with a mean of 0.88. By the end of forty-eight weeks, *Patients 570* and *585* had left the hospital, and the cytotoxic indices of the leukocytes from the remaining 4 patients ranged from 0.80 (*Patients 547*) to 0.98 (*Patient 551*), with a mean of 0.90. The cytotoxic indices at the end of fifty-four weeks ranged from 0.90 (*Patients 548*) to

[1] This study was supported by grant No. E-882 (C2R1) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service; and by a grant from the Kentucky Heart Association.

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[7] Scherago, M., *et al.*: Ann. Allergy, 1957, *15*, 1.

TABLE 1

CYTOTOXIC INDICES OF EIGHT PATIENTS AT SIX-WEEK INTERVALS DURING CHEMOTHERAPY USING OLD TUBERCULIN 1:100

Patient	Week										
	0	6	12	18	24	30	36	42	48	54	60
547	0.62	0.55	0.68	0.74*	0.80	0.76	0.85	0.88	0.80	0.94	0.89
548	0.53	0.60	0.63	0.75*	0.81	0.78	0.88	0.80	0.85	0.90	0.98
551	0.61	0.72*	0.68	0.78	0.75	0.84	0.88	0.94	0.98	0.96	0.90
573	0.64	0.69	0.60	0.80*	0.87	0.88	0.80	0.86	0.96	1.02	†
570	0.65	0.59	0.55	0.70	0.84	0.77*	0.83	0.90	†		
585	0.62	0.65	0.70	0.78*	0.90	0.85	0.81	0.92	†		
615	0.65	0.78	0.70	0.81*	0.91	0.84	0.93	†			
702	0.74	0.68	0.79*	0.88	0.94	0.86	0.90	†			
Mean cytotoxic index	0.63	0.66	0.67	0.78	0.85	0.83	0.86	0.88	0.90	0.95	0.92

* Sputum samples became negative for acid-fast bacilli.
† Patients had left the hospital.

1.02 (*Patient 573*), with a mean of 0.95. By the end of sixty weeks, *Patient 573* had left the hospital. The cytotoxic indices for the 3 remaining patients were 0.89, 0.98, and 0.90, with a mean of 0.92.

In every case the skin reaction was still positive when the last leukocytic test was made and was just as intense as before therapy.

* * *

If any cytotoxic index below 0.86 is considered to be indicative of specific tuberculin sensitivity (in other words a positive reaction) and any cytotoxic index above 0.95 as indicative of a lack of specific sensitivity (a negative reaction)[6] then all 8 patients had, on admission to the hospital, cytotoxic indices that were well within the range of specific leukocytic sensitivity indicative of active tuberculosis. It is also apparent that in 3 patients (*548*, *551*, and *573*), all with moderately active tuberculosis, the cytotoxic indices after sixty weeks of chemotherapy reached the range of nonspecific sensitivity associated with the absence of active tuberculosis. In all of the other patients there was a progressive decrease in sensitivity during chemotherapy. Unfortunately, 4 of the patients had left the hospital by the forty-eighth week. At the last testing before their departure their cytotoxic indices were 0.90 or more, and might well have reached the range of inactivity on subsequent testings. In *Patient 547*, the highest cytotoxic index obtained was 0.94 (by the fifty-fourth week), but it dropped to 0.89 by the sixtieth week.

The results, in general, agree with those which Hall and Scherago[3] obtained in their study of the effect of chemotherapy on the leukocytic sensitivity to tuberculin in guinea pigs experimentally infected with *Mycobacterium tuberculosis* H37Rv.

Should these findings prove to be reproducible on a larger group of patients, testing for leukocytic sensitivity could be used to determine progress of the disease toward the inactive state and the continuance of this state after the patient's discharge.

In summary: The *in vitro* leukocytic sensitivity to tuberculin of 8 patients with active pulmonary tuberculosis was determined at six-week intervals before and during chemotherapy.

The leukocytic sensitivity of 4 of the patients had decreased significantly within thirty-six and forty-two weeks before leaving the hospital; that of a fifth patient had decreased markedly at the end of sixty weeks; and that of the 3 remaining patients, to a nonspecific tuberculin sensitivity.

The mean cytotoxic indices for the group showed a progressive decrease in leukocytic sensitivity from admission to the termination of the experiment. The skin test reactions to PPD of all 8 patients remained positive throughout the study period.

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EFFECT OF PYRIDOXINE ON CONCENTRATIONS OF ACTIVE ISONIAZID IN SERUM

Peripheral polyneuritis has been the most frequently encountered toxic reaction occurring during therapy with isoniazid.[1, 2] However, psychoses,[2, 3] emotional disturbances,[4] optic neuritis,[7, 8] aggravation of grand mal epilepsy,[9-11] and aggravation of thrombocytopenic purpura[12] have been reported. These undesirable reactions have been encountered more frequently on regimens employing higher dosages of isoniazid.[2]

In 1953, Jones and Jones[13] suggested that deficiency of pyridoxine might be the etiologic factor of these peripheral neuropathies, as the utilization of this vitamin might be antagonized by isoniazid. Biehl and Skavlem,[14] and later Biehl and Vilter[1] demonstrated that the administration of isoniazid significantly increased the excretion of vitamin B_6 in the urine.

Thereafter, it became common practice to incorporate pyridoxine into high-dosage isoniazid regimens. Clinical trials proved the ability of pyridoxine to reduce the incidence of peripheral neuritis due to isoniazid administration.[14-16] The question whether the addition of pyridoxine to therapeutic regimens employing high dosages of isoniazid lessened the latter's antituberculosis effect has naturally been raised. It was shown that concentrations of pyridoxine far in excess of those achieved *in vivo* failed to modify the antimicrobial activity of isoniazid on tubercle bacilli *in vitro*.[17] Also, it was demonstrated that it did not affect the chemotherapeutic activity of isoniazid in laboratory animals challenged with standard strains of virulent tubercle bacilli.[18-20] However, a delayed isoniazid antagonism by pyridoxine was observed in mice given relatively huge dosages of this vitamin.[21]

The purpose of this investigation was to determine the effect of short and long courses of simultaneous administration of isoniazid and pyridoxine on the concentrations of antimicrobially active isoniazid in the serum of human subjects as determined by a microbiologic assay.

Nine adult tuberculous patients were selected for this study. For two days prior to the initial test the patients received 10 mg. of isoniazid per kg. of body weight twice daily. No other drugs were given. On the day of the test, a single oral dose of isoniazid, 10 mg. per kg. of body weight, was administered. The total amount given ranged from 450 to 800 mg. Venous blood samples for the determination of the concentrations of antimicrobially active isoniazid in the serum were obtained before the administration of the "loading" dose (zero-hour sample) and six hours after the administration of isoniazid (six-hour sample).

Repeat studies on each of these 9 patients were performed under the same conditions after administering the same amount of isoniazid and 200 mg. of pyridoxine daily for two days. The "loading" dose on the day of the test was 10 mg. of isoniazid per kg. of body weight, and 100 mg. of pyridoxine.

The possibility of delayed appearance of isoniazid antagonism by pyridoxine was investigated in a second group of 5 patients, who had been treated with 16 mg. of isoniazid per kg. of body weight (800 to 1,200 mg. daily), and 50 to 100 mg. of pyridoxine daily for periods of time ranging from two to eight months. The concentrations of antimicrobially active isoniazid achieved after a "loading" dose of 8 mg. of isoniazid per kg. of body weight at the time of the initiation of the

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isoniazid-pyridoxine treatment were compared with the concentrations achieved after the same "loading" dose of isoniazid two to eight months later.

The microbiologic assay for antimicrobially active serum isoniazid was performed as follows:

Preparation of serum samples: Eight milliliters of venous blood were collected aseptically from patients six hours following an oral loading dose equivalent to half the daily dosage of isoniazid.[22] The blood specimens were allowed to clot at room temperature, were centrifuged, the supernatant serum was transferred to sterile tubes and stored in the refrigerator. Serum has been stored for as long as twenty-one weeks without demonstrable change in drug concentration.

Inoculum: The inoculum used was a six- to seven-day-old stock culture of an isoniazid-susceptible, streptomycin-resistant (10 γ per ml.) strain of *Mycobacterium tuberculosis* (H37RvS-RSM), cultivated in Tween® 80-albumin standard transfer medium.[23] The organisms used were resistant to streptomycin in order to permit the simultaneous administration of streptomycin so that the concentrations of streptomycin could also be determined. The presence or absence of streptomycin from the serum did not alter the results of the microbiologic assay for isoniazid.[24]

Test medium: An oleic-acid–albumin medium was used.[23] The medium was seeded with the inoculum—4.4 ml. of inoculum in 110 ml. of medium.

Method of performing the assay: One milliliter of serum was distributed by serial twofold tube dilutions in seven tubes containing 2.5 ml. of the test medium seeded with 0.1 ml. of inoculum. The final serum dilutions ranged from 1:5 dilution in tube No. 1 to 1:320 dilution in tube No. 7. Standard controls containing known concentrations of isoniazid (0.0, 0.02, 0.04, and 0.08 γ per ml.) and the same inoculum were included with each set of determinations. The inoculated tubes and the standard controls were incubated for five to seven days at 36°C. Ziehl-Neelsen–stained smears were made from a loopful of contents of each tube and were examined for complete or partial loss of acid-fastness.[23]

Under these conditions, the controls consistently showed partial to complete loss of acid-fastness at an isoniazid concentration of 0.04 γ per ml., and complete loss at 0.08 γ of isoniazid per ml. The standard deviation of the results of fifty consecutive microbiologic assay tests with known concentrations of isoniazid provided an

TABLE 1

ISONIAZID CONCENTRATIONS DETERMINED BY BIOASSAY OF SERUM SIX HOURS AFTER A DOSE OF 10 MG. OF ISONIAZID PER KG. OF BODY WEIGHT WITHOUT AND WITH 100 MG. OF PYRIDOXINE (VITAMIN B_6)*

Patient	Isoniazid Concentration after 4 Mg. per Kg.	Isoniazid Concentration without Pyridoxine		Isoniazid Concentration with Pyridoxine	
		Zero-Hour Concentration†	Six-Hour Concentration‡	Zero-Hour Concentration	Six-Hour Concentration
		(γ per ml.)	(γ per ml.)	(γ per ml.)	(γ per ml.)
O.D.	0.2	<0.2	1.6	<0.2	1.2
R.C.	0.2	1.2	3.2	1.2	3.2
P.M.	0.4	0.4	1.2	0.4	1.6
C.J.	0.4	1.6	3.2	1.6	3.2
W.A.	0.4	1.6	4.8	1.6	3.2
R.S.	1.2	1.2	6.4	1.2	4.8
G.S.	1.2	1.6	4.8	1.6	3.2
M.O.	2.4	0.6	2.4	1.2	4.8
A.R.	2.4	2.4	4.8	2.4	4.8

* Patients received 20 mg. of isoniazid per kg. of body weight without or with 200 mg. of pyridoxine daily for two days prior to the test.

† Zero-hour concentration—18 hours after the last routine dose of isoniazid, blood was obtained and the test dose of isoniazid administered. Such zero-hour concentrations represent the moiety of active isoniazid still present.

‡ Six-hour concentration—blood was withdrawn six hours after the administration of the "loading" dose.

TABLE 2

ISONIAZID CONCENTRATIONS DETERMINED BY BIOASSAY OF SERUM SIX HOURS AFTER A "LOADING" DOSE OF 8 MG. OF ISONIAZID PER KG. OF BODY WEIGHT: COMPARISON OF VALUES OBTAINED AT TIME OF INITIATION OF HIGH-DOSAGE ISONIAZID-PYRIDOXINE REGIMEN, AND AFTER 2–8 MONTHS OF THERAPY

Patient	Isoniazid Concentration on Initiation of Chemotherapy	Duration of Chemotherapy prior to Repeat Study	Isoniazid Concentration at Time of Repeat Study
	(γ per ml.)	(months)	(γ per ml.)
D.A.	2.4	2	3.2
O.P.	3.2	3	2.4
B.H.	2.4	4	3.2
R.E.	3.2	4	2.4
M.W.	0.6	8	0.4

[22] The six-hour period after ingesting the "loading" dose of isoniazid was selected for the following reason: Careful serial determinations of antimicrobially active isoniazid have shown that the bimodal nature of the distribution of rapid and slow inactivators of isoniazid was not apparent at two hours, was poorly developed at four hours, and was clearly revealed at six hours.

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estimation of the accuracy of the microbiologic assay method: ±20 per cent.[24]

The concentration of antimicrobially active isoniazid in unknown specimens was estimated accordingly as follows:

	Isoniazid γ per Ml. of Serum
No loss of acid-fastness at 1:5 dilution	less than 0.2
Partial loss of acid-fastness at 1:5 dilution	0.2
Complete loss at 1:5, none at 1:10 dilution	0.3
Partial loss of acid-fastness at 1:10 dilution	0.4
Complete loss at 1:10, none at 1:20 dilution	0.6
Partial loss of acid-fastness at 1:20 dilution	0.8
Complete loss at 1:20, none at 1:40 dilution	1.2
Partial loss of acid-fastness at 1:40 dilution	1.6
Complete loss at 1:40, none at 1:80 dilution	2.4
Partial loss of acid-fastness at 1:80 dilution	3.2
Complete loss at 1:80, none at 1:160 dilution	4.8
Partial loss of acid-fastness at 1:160 dilution	6.4
Complete loss at 1:160, none at 1:320 dilution	9.6
Partial loss of acid-fastness at 1:320 dilution	12.8
Complete loss of acid-fastness at 1:320 dilution	more than 12.8

Using these criteria, the concentrations of isoniazid in the blood six hours after a "loading" dose of 4 mg. of isoniazid per kg. of body weight were determined on two separate occasions on 163 patients after one-day to six-month intervals.[24] The standard deviation of the second determination of the isoniazid concentration from the first determination on each patient throughout the range of 0.2 to 4.8 γ of isoniazid per ml. of serum was ±28 per cent.

The comparison of the serum concentrations of antimicrobially active isoniazid achieved by 9 tuberculous patients on a 10 mg. per kg. of body weight "loading" dose of isoniazid with and without pyridoxine did not reveal a difference between the paired levels greater than the error inherent in the technical procedure (table 1).

The concentrations achieved before and after a course of therapy of isoniazid and pyridoxine lasting from two to eight months were essentially unchanged in the 5 patients tested (table 2).

* * *

It has been known for some time that isoniazid is altered metabolically in the human body to chemical derivatives which have little or no antimicrobial activity.[25] It has also been recognized that the biochemical alteration of the isoniazid molecule occurs at widely different rates in different individuals.[26] However, the rate of inactivation was found to be constant in individual patients.[24] Thus the influence of other drugs on the concentration of antimicrobially active isoniazid in the blood could be tested by performing the tests on the same patients acting as their own controls.[24]

The purpose of this study was to find out whether pyridoxine influences the serum concentration of antimicrobially active isoniazid six hours after a "loading" dose. The serum level of isoniazid as estimated by microbiologic assay represents only the free, unaltered, or nonmetabolized portion of the drug, i.e., its microbiologically active moiety. The loss of acid-fastness by tubercle bacilli is caused only by isoniazid—no other known antimicrobial drug has such an effect.[27]

In summary: The results of this study clearly demonstrated that the dosage of pyridoxine usually employed in clinical practice[15] did not influence the six-hour serum concentration of that moiety of isoniazid that has a therapeutic action. The results were the same after a short course or after a prolonged course of therapy with both drugs.

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VIII. KANAMYCIN IN THE TREATMENT OF PULMONARY TUBERCULOSIS, INCLUDING STUDIES OF DRUG TOXICITY[1]

(A Report of the Cooperative Study by Veterans Administration Hospitals)

The isolation of kanamycin from *Streptomyces kanamyceticus* was announced by Hamao Umezawa in 1957.[2] It was found to be a potent drug, active against many microorganisms, including tubercle bacilli.[3] This activity was found to be present against tubercle bacilli which had become resistant to many of the common antituberculosis drugs.[4] These included PAS, cycloserine, streptovaricin, streptomycin, and isoniazid. On the other hand, strains of tubercle bacilli resistant to neomycin and viomycin were not inhibited by kanamycin, although kanamycin-resistant strains were inhibited by viomycin. Moreover, when kanamycin-susceptible tubercle bacilli were grown in increasing concentrations of this drug, resistance occurred rather promptly.

Kanamycin is water soluble and when injected intramuscularly is excreted in the urine in an unchanged form.[5] It is not absorbed from the gastrointestinal tract.[6] Renal and auditory toxicity are known to occur but, from animal experimentation, it was thought that this might be somewhat less than that expected from streptomycin or dihydrostreptomycin.[5]

A small cooperative study of the toxicity and the therapeutic effectiveness of kanamycin was set up by the U. S. Veterans Administration–Armed Forces. Tuberculous patients with cavitary pulmonary disease with tubercle bacilli in the sputum, whose treatment had been unsuccessful on other chemotherapeutic regimens, were the subjects of the investigation. The emergence of tubercle bacilli resistant to kanamycin, and possible delay in the appearance of kanamycin-resistant tubercle bacilli when PAS or tetracycline phosphate complex (TCPC) was added, were also studied. Patients with known renal damage were to be excluded, as well as patients whose tubercle bacilli were known to be resistant to viomycin and neomycin. The study was divided into two parts: first, a study composed of patients whose tubercle bacilli were known to be resistant to streptomycin, isoniazid, and PAS; and second, one in which the organisms were known to be resistant to streptomycin and isoniazid, but were susceptible to PAS. Patients received drug regimens according to a centrally controlled envelope method of random allocation.

In the first study, patients received kanamycin alone, 1 gm. daily (0.5 gm. twice daily intramuscularly); or kanamycin, 1 gm. daily (0.5 gm. twice daily intramuscularly) with tetracycline phosphate complex, 4 gm. daily (1 gm. four times a day by mouth).

In the second study, the patients received kanamycin alone, 1 gm. daily (0.5 gm. twice daily intramuscularly); or kanamycin, 1 gm. daily (0.5 gm. twice daily intramuscularly), and PAS, 12 gm. a day (4 gm. a day three times a day by mouth). The regimens were to be maintained for six months, unless interruption or termination was required by unfavorable progress or toxic manifestations.

In addition to the usual roentgenographic and bacteriologic studies and routine laboratory safeguards of toxicity, audiograms were to be performed prior to and every two weeks during therapy. In the first study, 28 patients received kanamycin alone, while 27 received it with tetracycline phosphate complex. In the second study, 11 patients received kanamycin alone and 9 received it with PAS.

The Veterans Administration hospitals which participated in the study and supplied case histories for this report were Butler, Pennsylvania; Castle Point, New York; New Haven, Connecticut; Sunmount, New York; and Oteen, North Carolina. Most of the cases were started under treatment in June or July, 1958.

Drug toxicity: Treatment was stopped for a variety of reasons in 65 of the 75 patients by the end of January, 1959. The principal form of drug toxicity encountered was deafness, which occurred in 29 (39 per cent) of those beginning treatment. The deafness often was severe, and audiograms usually were unnecessary to detect it. In at least one case, deafness continued to progress after treatment was stopped. Three patients developed hearing loss by the end of the first month of treatment, 6 in the second month, 6 in the third month, 2 in the fourth month, 9 in the fifth month, and 3 in the sixth month. The audiographic data

[1] Presented in summary form at the Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces, February 3–6, 1959, St. Louis, Missouri.

[2] Umezawa, H., *et al.*: J.Antibiot. (Japan), 1957, *10*, 181.

[3] Umezawa, H.: Ann. New York Acad. Sc., 1958, *76*, 20.

[4] Steenken, W., Jr., *et al.*: Ann. New York Acad. Sc., 1958, *76*, 103.

[5] Tisch, D. E., *et al.*: Ann. New York Acad. Sc., 1958, *76*, 44.

[6] Cohn, I., Jr.: Ann. New York Acad. Sc., 1958, *76*, 212.

TABLE 1

FIRST STUDY: DISAPPEARANCE OF TUBERCLE BACILLI FROM SPUTUM AS DETERMINED BY MICROSCOPY AND CULTURE

Months	Pre-treatment	1	2	3	4	5	6
Kanamycin alone							
Microscopy positive	26	19	17	13	6	5	2
Microscopy negative	2	7	7	4	5	3	1
Culture positive	24	23	18	14	10	5	2
Culture negative	4	3	2	0	0	0	0
Kanamycin and tetracycline phosphate complex							
Microscopy positive	23	16	10	5	4	3	1
Microscopy negative	4	10	9	8	4	2	0
Culture positive	23	21	17	9	6	1	1
Culture negative	4	2	0	2	1	0	0

TABLE 2

SECOND STUDY: DISAPPEARANCE OF TUBERCLE BACILLI FROM SPUTUM AS DETERMINED BY MICROSCOPY AND CULTURE

Months	Pre-treatment	1	2	3	4	5	6
Kanamycin alone							
Microscopy positive	8	5	5	4	2	3	0
Microscopy negative	3	3	2	2	2	1	0
Culture positive	9	8	7	4	4	2	0
Culture negative	2	1	0	0	0	0	0
Kanamycin and PAS							
Microscopy positive	8	3	6	5	2	2	0
Microscopy negative	1	2	1	0	1	1	0
Culture positive	9	6	6	2	2	0	0
Culture negative	0	0	1	1	0	1	0

prior to treatment were not available in all cases. However, of 7 who became deaf under treatment, 3 had normal findings, and 4 evidence of slight hearing loss in pretreatment audiograms. Other eighth-nerve toxicity was uncommon. Only 4 patients complained of vertigo, one of whom also became deaf. The proportion of patients becoming deaf was the same whether they received kanamycin alone or PAS or tetracycline phosphate complex as well.

Drug allergy, presumably due to kanamycin, occurred in one case. Only one instance of monilia infection of the mouth occurred in the patients receiving tetracycline phosphate complex. Gastrointestinal disturbance from this drug made cessation of treatment necessary in only 3 cases. While complete data were not available, it was believed that many instances of gastrointestinal intolerance to 4 gm. of tetracycline phosphate complex occurred and that the dose was reduced frequently to 1 or 2 gm. a day. In only 2 cases was the treatment stopped due to renal toxicity after two months, although the finding of casts in the urine occurred very commonly. Pain at the site of injection, although commonly complained

TABLE 3

FIRST STUDY: KANAMICIN-RESISTANT TUBERCLE BACILLI AS DETERMINED BY EQUAL GROWTH IN DRUG-CONTAINING AND DRUG-FREE MEDIUM

Months	Pre-treatment	1	2	3	4	5	6
Kanamycin alone							
Susceptible	18	11	9	8	3	3	0
10 γ per ml.	2	1	1	0	0	0	0
50 γ per ml.	0	0	1	1	1	1	0
100 γ per ml.	0	0	1	1	2	0	1
Total culture positive	24	23	18	14	10	5	2
Kanamycin and tetracycline phosphate complex							
Susceptible	18	12	7	3	2	0	0
10 γ per ml.	0	2	2	3	2	0	0
50 γ per ml.	0	0	0	0	0	0	0
100 γ per ml.	0	0	0	1	1	1	0
Total culture positive	23	21	17	9	6	1	1

TABLE 4

SECOND STUDY: KANAMYCIN-RESISTANT TUBERCLE BACILLI AS DETERMINED BY EQUAL GROWTH IN DRUG-CONTAINING AND DRUG-FREE MEDIUM

Months	Pre-treatment	1	2	3	4	5	6
Kanamycin alone							
Susceptible	7	7	4	1	2	0	0
10 γ per ml.	0	0	0	0	1	0	0
50 γ per ml.	0	1	1	0	0	1	0
100 γ per ml.	0	0	2	2	1	1	0
Total culture positive	9	8	7	4	4	2	0
Kanamycin and PAS							
Susceptible	7	3	6	1	1	0	0
10 γ per ml.	0	2	0	0	1	0	0
50 γ per ml.	0	0	0	1	0	0	0
100 γ per ml.	0	0	0	0	0	0	0
Total culture positive	9	6	6	2	2	0	0

of by patients, made it necessary to stop treatment in only 5 cases.

Therapeutic effectiveness: Treatment was stopped due to unfavorable roentgenographic change in 3 cases. There were 3 deaths, 2 from progressive tuberculosis, and one following a massive hemoptysis. Eight patients were lost by premature discharge from the hospital. The treatment of the remainder was stopped because it was believed that no particular result had been attained, and it was believed that the risk of auditory toxicity was too great to justify continuing what had proved to be an ineffective regimen in these particular cases.

No attempt was made to evaluate favorable roentgenographic change; however, it was of some interest to find that evidence of unfavorable progress as seen in the roentgenogram was relatively inconspicuous. Such progression of disease occurred in 6 patients who received kanamycin alone, in one patient who received kanamycin and tetracycline phosphate complex, and in 2 who received kanamycin and PAS.

No real difference in the reversal of infectiousness could be found when comparing the results attained by kanamycin alone with either kanamycin and PAS, or kanamycin and tetracycline phosphate complex (tables 1 and 2). The sputum "conversion rate" as determined microscopically was low and almost nonexistent by culture. This was an expected finding when it is considered that the case material was made up of treatment failures on other regimens.

The persistence of infectious sputum made the cases all the more valuable from the point of view of the study of the development of bacterial resistance to the drugs involved. Resistance data in both studies were tabulated, using growth equal to that of the control tube as indicating resistance to that concentration of drug (tables 3 and 4). Unfortunately, drug susceptibility was not determined on all cultures. The data available were much too small for any definite conclusions concerning the role of PAS or tetracycline phosphate complex in delaying the appearance of kanamycin-resistant tubercle bacilli. Data on PAS resistance were available in very few cases. Insofar as was known, all but one patient-strain showed complete susceptibility to PAS prior to treatment. In only 2 cases was there a change from this state in that definite growth of tubercle bacilli, although less than in the control culture, occurred in the 10-γ drug concentration after two and three months of treatment, respectively.

Data on the emergence of resistance to tetracycline phosphate complex were available in 10 cases. The pretreatment drug susceptibility varied from 10 to 20 γ per ml., and resistance to the 20-γ concentration of drug was not exceeded in any case in which a patient was receiving from one to four months of treatment.

* * *

Japanese investigators[7] have reported that a dosage of 6 gm. or more of kanamycin a week was attended by an appreciable incidence of eighth-nerve toxicity but, when lesser amounts were used, deafness was rare. They also concluded that less than 4 gm. a week was likely to result in poor therapeutic effects. They recommended a regimen which they followed in the majority of their cases, consisting of 2 gm. of kanamycin daily given in two divided doses, morning and evening, twice weekly, thus making a total of 4 gm. a week.

The occurrence of deafness in 29 of 75 patients within six months of beginning treatment is a matter of very considerable significance in the evaluation of kanamycin. This frequency of ototoxicity has been only approximately equaled, at least in one of the hospitals (Oteen) studying the drug when, a number of years ago, a few patients suffering from tuberculous meningitis received 4 gm. of dihydrostreptomycin daily as a single drug for several months. Although other forms of toxicity due to kanamycin were relatively benign and infrequent, 65 of the 75 patients entering the study properly had treatment stopped because the expectation of deafness outweighed the prospect of a worthwhile therapeutic result.

Thus, it has been shown by this Veterans Administration–Armed Forces study that the ototoxicity of kanamycin was prohibitive when used in a dose of 1 gm. daily, and that a much lower dose would be necessary for safe long-term therapy.

In summary: Seventy-five previously treated tuberculous patients with cavitary pulmonary disease and tubercle bacilli in the sputum received 1 gm. of kanamycin daily alone, with PAS, or with tetracycline phosphate complex; they were assigned to the different regimens by random allocation. While there was some evidence of therapeutic efficacy, the incidence of deafness due to kanamycin (39 per cent of patients beginning treatment) prohibited the further employment of this drug in the dosage used.

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May 18, 1959

[7] Committee of Chemotherapy for Tuberculosis, Japan: Ann. New York Acad. Sc., 1958, *76*, 166.

AN INVESTIGATION OF THE RELIABILITY OF THE NIACIN TEST, THE NEUTRAL RED TEST, AND THE TEST FOR CORD FORMATION FOR DIFFERENTIATION OF GROUP III ("BATTEY" STRAINS) OF ATYPICAL MYCOBACTERIA FROM HUMAN TUBERCLE BACILLI

The increasing number of reports appearing in the literature in recent years indicates that widespread interest is being focused on the so-called atypical mycobacteria. This interest is with particular reference to their classification, identification, and pathogenicity to man.[1-7]

At the present time, however, the classification of the atypical mycobacteria is incomplete. They are divided into four broad groups on the basis of growth characteristics, cellular and colonial morphology, and development or change in colony pigment after exposure to light.[8] With regard to pathogenicity to man, the present knowledge may be briefly summarized as follows:

Group I, or photochromogens, are associated with pulmonary disease, the clinical course of which is chronic, indolent, and refractory to treatment. *Group II*, or scotochromogens, are recovered from patients demonstrating chronic pulmonary disease who have been discharging tubercle bacilli prior to the isolation of the other mycobacteria. *Group III*, "Battey" or nonphotochromogenic strains, are associated with acute pulmonary disease indistinguishable from that produced by human tubercle bacilli but more indolent and refractory to treatment. *Group IV* consists of the rapid-growing atypical strains, some of which have been credited with being the sole infectious agents of severe pulmonary disease.

As acute pulmonary infection caused by Group *III* atypical mycobacteria is indistinguishable from that produced by human tubercle bacilli, it becomes essential that cultures of the former be recognized and identified in the routine laboratory. Although these acid-fast bacilli are adequately defined with respect to their morphologic and colonial characteristics, strains are occasionally encountered that cannot be distinguished with certainty, by the above characteristics alone, from human tubercle bacilli. Therefore, a study was undertaken to determine whether or not certain other characteristics could serve as reliable supplementary means of differentiation.

Source of cultures: One hundred and fifteen strains of *Group III* atypical mycobacteria were cultured from various types of diagnostic material (from hospitalized patients), sent to the tuberculosis laboratory for culture of *M. tuberculosis*. These strains were identified as such on the basis of growth requirements and cellular and colonial appearance as defined in the *Manual of Tuberculosis Laboratory Methods* of the Veterans Administration–Armed Forces, June, 1958; 18 strains of human tubercle bacilli cultured from 4 patients having pulmonary tuberculosis; and 2 control strains, one each of H37Rv and H37Ra (human) obtained from the Trudeau Laboratory, Saranac, New York. All strains (133 in number) cultured from hospitalized patients were initially isolated on Löwenstein-Jensen medium prepared in this laboratory.

Smear preparations for microscopic examination were stained by the Ziehl-Neelsen technique.

Laboratory tests: The direct qualitative chemical method for determination of niacin content, as described by Konno,[7] was employed for differentiation of human tubercle bacilli from *Group III* atypical mycobacteria. The neutral red test[9] and the test for cord formation[10] were used for virulence studies.

All 135 strains were tested for their ability to produce a positive niacin reaction, whereas virulence studies were limited to 46 of the *Group III* strains and to the 20 strains of human tubercle bacilli. The former were selected, on the basis of their representing multiple recoveries, from diagnostic material (in the absence of recovery of human tubercle bacilli) from 8 patients having clinical and roentgenographic evidence of pulmonary disease comparable with pulmonary tuberculosis.

A comparison of the results of the niacin test in relation to the two types of bacilli studied is presented in table 1.

[1] Runyon, E. H.: M. Clin. North America, 1958, *43*, 273.

[2] Engbaek, H. C., *et al.*: Acta tuberc. scandinav., 1957, *34*, 145.

[3] Schmidt, L. H., *et al.*: Am. Rev. Tuberc., 1957, *75*, 169.

[4] Wolinsky, E., *et al.*: Am. Rev. Tuberc., 1957, *75*, 180.

[5] Crow, H. E., *et al.*: Am. Rev. Tuberc., 1957, *75*, 199.

[6] Hardy, A. V., *et al.*: Am. J. Pub. Health, 1958, *48*, 754.

[7] Konno, K., *et al.*: Am. Rev. Tuberc., 1958, *77*, 669.

[8] *Manual of Tuberculosis Laboratory Methods*, Veterans Administration–Armed Forces, June, 1958, p. 15.

[9] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tuberc., 1948, *58*, 698.

[10] Middlebrook, G., *et al.*: J. Exper. Med., 1947, *86*, 175.

Of the 135 strains tested, 22, or 16 per cent, produced atypical niacin reactions. Of the 115 *Group III* strains, 16, or 13 per cent, produced atypical reactions. Of the 20 strains of human tubercle bacilli, 6 produced atypical reactions including the avirulent control strain H37Ra.

By "atypical" reaction is meant either that a *Group III* strain produced a positive niacin test, or human tubercle bacilli produced a negative niacin test.

A comparison of the results of the niacin test, the neutral red test, and the test for cord formation in relation to the two types of bacilli studied is presented in table 2.

Niacin test (direct chemical test): Of the 46 *Group III* strains tested, 5, or 10 per cent, produced atypical reactions. As previously mentioned, 6 of the 20 strains of human tubercle bacilli produced atypical reactions.

Neutral red test: All 46 *Group III* strains and the 19 strains of virulent human tubercle bacilli were able to bind the dye neutral red. The avirulent control strain H37Ra was not able to bind the dye.

Cord formation: Eighty-four per cent of the *Group III* strains demonstrated "cording" to a slight degree in liquid medium. The 19 strains of virulent human tubercle bacilli demonstrated their ability to form "tight" or "serpentine" cords. Cultures of the avirulent control strain H37Ra in liquid medium failed to demonstrate cord formation.

* * *

Konno[7] has reported that the difference in niacin production between human tubercle bacilli and atypical acid-fast bacilli is a reliable index for identification of the human bacilli. In the present

TABLE 1

RESULTS OF THE NIACIN TEST IN RELATION TO GROUP III ATYPICAL MYCOBACTERIA AND HUMAN TUBERCLE BACILLI
(135 Strains)

Species	Total Number	Source	Niacin Test	
			Number Positive	Number Negative
Group III atypical mycobacteria	82	Sputum	12	70
	28	Gastric lavage	1	27
	4	Urine	3	1
	1	Bronchial secretion	0	1
Human tubercle bacilli	14	Sputum	11	3
	2	Gastric lavage	2	0
	1	Chest drainage	0	1
	1	Surgical tissue	0	1
Controls (human strain)				
H37Rv	1		1	0
H37Ra	1		0	1
Totals	135		30	105

TABLE 2

RESULTS OF THE NIACIN TEST, THE NEUTRAL RED TEST, AND THE TEST FOR CORD FORMATION IN RELATION TO GROUP III ATYPICAL MYCOBACTERIA AND HUMAN TUBERCLE BACILLI
(66 Strains)

Species	Total Number	Source	Niacin Test		Neutral Red Test		Test for Cord Formation	
			+	−	+	−	+	−
Group III atypical mycobacteria	21	Sputum	4	17	21	0	19*	2
	24	Gastric lavage	1	23	24	0	20*	4
	1	Bronchial secretion	0	1	1	0	1*	0
Totals	46		5	41	46	0	40*	6
Human tubercle bacilli	15	Sputum	11	4	15	0	15	0
	2	Gastric lavage	2	0	2	0	2	0
	1	Chest drainage	0	1	1	0	1	0
Control strains:								
H37Rv	1		1	0	1	0	1	0
H37Ra	1		0	1	0	1	0	1
Totals	20		14	6	19	1	19	1

* Slight cording

study, this direct chemical test for niacin production was employed to ascertain whether it would reliably serve to differentiate between human tubercle bacilli and, specifically, *Group III* atypical mycobacteria.

In the analysis of the data, two main points were encountered which stand out in sharp contrast to the results obtained by Konno.[7] First, it was not possible to obtain consistent results. Atypical reactions occurred among cultures of both types of bacilli, including the avirulent control strain H37Ra (human type). As this test presumably serves for differentiating human tubercle bacilli from all other types of acid-fast bacilli, and is in no way related to virulence, the avirulent control strain should have produced a positive niacin reaction. Second, it was not possible to obtain clean-cut results. In addition to cultures developing a definite yellow color (indicating a positive niacin test), cultures were encountered among both types of bacilli, developing colors ranging from all shades of blue through all shades of green, some having a slight yellow cast. This phenomenon made interpretation of results extremely difficult. After much consideration (and retesting of such cultures, in which primary results were always reproducible), it was decided to consider only those cultures which developed a *definite yellow* color as having the ability to produce a positive niacin reaction. It is on this basis of interpretation that the incidence of 16 per cent of atypical reactions was calculated. Therefore, it is obvious that the direct chemical test for niacin production was found to be difficult to read and unreliable for use as a diagnostic tool to distinguish between human tubercle bacilli and *Group III* atypical mycobacteria.

Another point that stands out in the analysis of these data is the persistent binding of the dye neutral red by the 46 strains of *Group III* acid-fast bacilli as well as by the 19 strains of virulent human tubercle bacilli. Such results suggest a possible metabolic link between these two types of bacilli and, in view of the fact that all *Group III* strains were recovered from patients having evidence of acute pulmonary disease, a possible pathologic link as well. It is obvious that the neutral red test cannot serve to differentiate between these two types of acid-fast bacilli.

With regard to the results of the tests for cord formation, although 84 per cent of the *Group III* strains demonstrated cording to a slight degree in liquid medium, none demonstrated the ability to form "tight" or "serpentine" cords, as did the 19 strains of virulent human tubercle bacilli. Therefore, this test could serve as a supplementary procedure to differentiate between these two types of bacilli provided the term "cord formation" is defined as: "the ability of a given strain to form microscopic cords of varying thickness and length consisting of bacilli oriented in parallel along the cords."

In summary: Of the three laboratory procedures investigated for differentiating between *Group III* atypical mycobacteria and virulent human tubercle bacilli, only the test for demonstration of "serpentine" cord formation proved of value. Results of the direct test for niacin production[2] were too inconsistent to warrant the use of this test for the above-stated purpose, and both types of acid-fast bacilli were able to bind the dye neutral red.

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June 22, 1959

[11] The writers wish to acknowledge the valuable technical assistance of Abraham Resnick.

Correspondence

RESULTS OF THE PAS URINE TEST (PHENISTIX) STUDY DONE IN MAY AND JUNE, 1959

To the Editor of the American Review of Respiratory Diseases:

The results of the PAS urine test (Phenistix) done during May and June, 1959, on ambulatory tuberculous patients for whom PAS was prescribed by the 25 clinics of the Bureau are shown in table 1.

The table gives the results by clinic and is self-explanatory. Positive urine tests for PAS were noted in 60.5 per cent of the total of 2,622 patients tested, with the percentages of positive tests ranging from 46 to 75 in the various clinics. Of the patients classified as taking PAS regularly, 77.8

TABLE 1

RESULTS OF PAS URINE TEST (PHENISTIX) MAY—JUNE, 1959

Bureau of Tuberculosis Chest Clinics

Name of Clinic	Totals						Result by Special Groupings											
	Total Number of Tests Done		Over-all Results				PAS Taken						Hour Interval between Last PAS Dose and Test					
			Positive		Negative		Regularly		With Occasional Lapses		Irregularly or Not at All		0–12		13–24		More than 24	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Astoria	53	100.0	35	66.0	18	34.0	33	8	2	1	—	9	35	8	—	1	—	9
Bedford	278	100.0	206	74.1	72	25.9	186	30	18	22	2	20	185	29	18	26	3	17
Brownsville	58	100.0	39	67.2	19	32.8	39	15	—	—	—	4	37	9	2	5	—	5
Bushwick	51	100.0	35	68.8	16	31.4	31	6	4	4	—	6	34	7	1	—	—	9
Central	104	100.0	65	62.5	39	37.5	61	22	3	10	1	7	59	15	5	11	1	13
Chelsea	205	100.0	103	50.2	102	49.7	96	60	6	29	1	13	96	66	5	20	2	16
Corona	38	100.0	27	71.0	11	29.0	26	9	1	2	—	—	27	5	—	3	—	3
Crown Heights	132	100.0	66	59.0	56	41.0	59	32	6	18	1	6	58	26	4	18	4	12
East Harlem	67	100.0	41	61.2	26	38.8	38	10	2	8	1	8	40	9	1	—	—	17
Ft. Greene	151	100.0	85	56.3	66	43.7	74	30	10	17	1	19	81	32	3	9	1	25
Lower Harlem	130	100.0	63	48.4	67	51.6	61	55	2	4	—	8	59	50	1	11	3	6
Manhattanville	154	100.0	79	51.3	75	48.7	71	20	4	20	4	35	68	35	8	10	3	30
Morrisania	184	100.0	104	56.5	80	43.5	80	44	23	24	1	12	93	40	9	21	2	19
Montefiore	103	100.0	66	64.1	37	35.9	60	29	4	7	2	1	58	23	5	8	3	6
Mott Haven	132	100.0	68	51.5	64	48.5	61	8	7	45	—	11	68	22	—	28	—	14
Red Hook	58	100.0	32	55.2	26	44.8	26	12	6	6	—	8	29	17	1	3	2	6
Richmond	39	100.0	28	71.7	11	29.3	26	2	2	7	—	2	28	2	—	5	—	4
Rockaway	33	100.0	22	66.6	11	33.3	17	3	5	6	—	2	21	5	1	3	—	3
Sunset Park	60	100.0	45	75.0	15	25.0	41	6	3	4	1	5	30	7	14	4	1	4
Tremont	123	100.0	70	57.0	53	43.0	65	32	5	16	—	5	62	31	6	13	2	9
Triboro	158	100.0	123	78.0	35	22.0	99	13	22	15	2	7	109	15	10	5	4	15
Upper Harlem	37	100.0	17	46.0	20	54.0	15	12	2	3	—	5	16	13	1	2	—	5
Washington Hts.	149	100.0	78	52.4	71	47.6	70	26	8	31	—	14	68	25	8	13	2	33
Wmsbg.-Grnpt.	135	100.0	90	66.6	45	33.3	81	18	9	17	—	10	86	23	3	7	1	15
Totals	2,622	100.0	1,587	60.5	1,035	39.5	1,416	502	154	316	17	217	1,447	514	106	226	34	295
Per cent within each category							77.8	22.2	34.9	65.1	7.3	92.7	73.7	26.3	31.9	68.1	10.3	89.7
							100.0		100.0		100.0		100.0		100.0		100.0	

per cent had positive tests; of those who were alleged to have taken PAS within twelve hours of the test, 73.7 per cent had positive tests. Thus, although a careful history can reveal quickly that patients are not taking the drug, it requires supplementation by urine testing to confirm a statement by the patient that PAS is being taken regularly. Most patients tell the truth—but at least 20 per cent of this clinic population do not.

Not indicated in the table is the difference in results in patients listed as "active" or "non-active." Of 626 patients designated as having active tuberculosis, 421, or 67.3 per cent, had positive test reactions to PAS. Of the 1,773 patients designated as having non-active tuberculosis, 1,035, or 58.4 per cent, had positive test reactions to PAS in the urine. In 223 instances, no diagnosis was indicated, and 131, or 59.0 per cent, of these patients had positive test reactions to PAS. This suggests that, as a whole, the active patients are taking their PAS a little more regularly than the non-active ones.

These results emphasize the importance of taking a careful history on drug acceptance in all patients for whom PAS (or isoniazid) is prescribed. A urine test for PAS should be done two or three times a year on patients who claim they are taking this drug. Proper corrective action should be taken as soon as it has been determined, by history or urine test, that a patient has not been taking his prescribed medication regularly.

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September 3, 1959

SURGERY IN TUBERCULOSIS

To the Editor of the American Review of Respiratory Diseases:

In a recent article,[1] Drs. Mitchell and Auerbach reviewed their thoughts regarding surgery in pulmonary tuberculosis and concluded that there is practically no need for surgery in "negative" cases. At the same time, they advise that contemplated surgery may be considered in the first several months of treatment rather than waiting for the usual six months or more. One might even infer facetiously that, although properly treated cases will always become negative, and negative cases need no surgery, one should operate early before the patient becomes negative. Although the article contains a number of topics with which one might take issue, we would like to comment chiefly upon the philosophy of treatment and resectional surgery in the "negative" cases as stated by the authors.

In any communicable disease, the ideal therapeutic aim is twofold—to render the patient's disease noncommunicable to others, and to produce such good healing that the patient will never have a relapse and endanger himself and others.

To render a tuberculous patient negative is relatively easy in this chemotherapeutic age, but to produce true pathologic healing is a much more difficult goal to achieve, as evidenced by bacteriologic and pathologic examination of pulmonary tissue resected many months after attaining a clinically negative bacteriology. During this period of negativity, the patient may be "healthy" in that he feels "well" and can even work, but as long as he carries active foci of disease he may reactivate. And our best follow-up studies are only several years old, a period of time that was considered inadequate even in pre-drug days.

How good a therapeutic result is desired? Since the present drugs are not the ultimate sterilizing agents and so long as active caseous foci and tubercle bacilli persist in the body, there remains an ever present danger of reactivation from various physical, chemical, and emotional stresses. One can formulate at least three goals in our aim of a "tuberculosis cure." The ideal goal is the death of all bacilli, the resolution or fibrosis of all inflammatory foci, and perhaps even a reversion of the tuberculin skin test. Such a cure would be analogous to that produced by penicillin in early syphilis; unfortunately, in tuberculosis, it is rare indeed and may occur only in a few recent converters detected and treated immediately. The second best result would include the rendering of the patient negative bacteriologically and removing all large necrotic foci which have been so notorious in producing relapse. The third best result would be to produce a negative sputum and reduce pathologic activity to such a degree that drugs plus natural forces might keep the disease under some degree of control.

The level of "cure" attainable and the place of surgery in the therapeutic plan depend upon a number of factors, such as the following:

(*1*) The physician—his philosophy, experience, and judgment with respect to closed lesions, open lesions, public health, rehabilitation.

(*2*) The person who has the disease—his aim, cooperation, and understanding; the presence of personality disorder, alcoholism, jail history, and recalcitrancy; and the time

[1] MITCHELL, R. S., AND AUERBACH, O.: Am. Rev. Resp. Dis., 1959, *80*, 207.

he is willing to spend to attain the desired result.

(3) The disease—acute or chronic, localized or disseminated, original or relapsing.

(4) How good is the available surgery? Even the mentioned 10 per cent relapse may be a higher figure than the complication rate in trained hands.

It may be argued that those who do not use surgery at all are the "therapeutic radicals."

Those who advocate that surgery has a definite place in treatment have never entertained the absurd notion that pulmonary resection can eradicate such a generalized disease. The only claim made was that the removal or collapse of large necrotic foci and cavitary areas might prevent future relapses, especially in cases which had not responded to chemotherapy as well as was expected.

If all patients were seen early and cooperated during their original course of treatment, relatively little surgery would be necessary. (In order to impress this point on our surgical residents, a number of such patients are assigned to their care.) However, the picture is not so encouraging in many hospitals. In our own institution, for example, the type of disease is well shown by the fact that 78 per cent of the patients have advanced lesions, 36 per cent have had the disease over five years, and 56 per cent are readmissions. The type of patient is shown by a 36 per cent incidence of premature discharges, 45 per cent alcoholism, and a 24 per cent history of jail residence. In such patients with such disease, the drug failure rate is high and a situation exists similar to that of pre-chemotherapy days. Judicious surgery is then indeed an important therapeutic tool.

The inference that clinically negative disease may be regarded as an adequate cure is not borne out too well in our own experience. In one of our series[2] of resections on 31 patients who had been clinically negative for an average of ten months and were systemically "well," there were 24 blocked cavities up to 4 cm. in diameter and 7 large caseous masses; visible bacilli were present in all of them and culturable bacilli in 22 per cent.

With regard to "open-negative" cavities, the Mitchell-Auerbach article states that 10 per cent to 20 per cent of such cases show true healing; in other words, 80 per cent to 90 per cent do not show such healing. On "re-examining" our last 25 consecutive resections for open-negative cavities, tubercle bacilli were found on smear or culture in 23 cases, or 92 per cent. In considering open healing, one must be sure to exclude large emphysematous bullae and cysts which are not healed tuberculous cavities even though they may be surrounded and even ulcerated by caseous masses in the vicinity. Furthermore, it is often impossible to differentiate clinically between a cyst and a tuberculous cavity. To be able to answer the question of how many tuberculous cavities can truly heal in open fashion, we will need many more years of observation and anatomic study following resection.

Undoubtedly, open-negative cavities are less dangerous than their positive counterparts, but the danger of relapse is always present if we expect to subject the patient to greater longevity, the stresses and strains of civilization, and grandchildren. This is particularly true of the uncooperative patient and especially after he leaves the hospital. By no means do we advocate surgery on all such cases, for there are contraindications present in many of them. We do stress, however, the need for judicious surgery in the patients at the levels of cure below the ideal goal mentioned above.

There are at least two other items in the Mitchell-Auerbach article with which one may take issue. One deals with the statement that a "phenomenon of modern chemotherapy is the apparent failure of large cavities to respond to collapse." Such has not been our experience. In fact, we feel that our collapse results have been superior to those of the pre-chemotherapy era, especially if the thoracoplasty or extraperiosteal plombage has been properly performed.

The second item deals with their views on bronchiectasis (not foreshortened bronchi). To their biased bibliography, we would like to add one of numerous articles proving the existence of tuberculous bronchiectasis in upper lobes.[3] However, discussion on these topics can await another occasion.

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September 25, 1959

[2] SALKIN, D.: Tuberculology, 1958, *17*, 54.

[3] SALKIN, D.: California Med., 1950, *73*, 577.

Books

THE YEAR BOOK OF MEDICINE (1958-1959 Year Book Series). Edited by Paul B. Beeson, Carl Muschenheim, William B. Castle, Tinsley R. Harrison, Franz J. Ingelfinger, and Philip K. Bondy. Pp. 782, The Year Book Publishers, Chicago, Illinois, 1958. Price $7.50.

This excellent volume represents the latest publication in a series that probably is the best attempt yet made to help physicians to cope with the immense quantities of literature germane to internal medicine which are published annually. The papers to be abstracted are selected by eminent clinicians and investigators, who frequently add their own enlightening comments to the well-done abstracts. The choice of papers reviewed is excellent, and covers most of the year's important work pertaining to internal medicine from the American, British, and (to a small extent) French literature. Pertinent illustrations and graphs are well reproduced.

One drawback, almost inevitable in a summary volume, is exemplified by the fact that most of the papers abstracted in the 1958-1959 Year Book appeared in 1957, although a few were initially published in early 1958. The time lag is not a critical defect, however, and the immense over-all worth of the volume leads this reviewer to commend it highly.

JEREMIAH A. BARONDESS
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THE PNEUMOCONIOSIS PROBLEM, WITH EMPHASIS ON THE ROLE OF THE RADIOLOGIST. By Eugene P. Pendergrass. Pp. 146, Charles C Thomas, Springfield, Illinois, 1959. Price, $6.75.

This small book with an ambitious title is intended primarily to arouse the interest of roentgenologists in the pneumoconioses, and to present guide lines which the author has found helpful in the interpretation of shadow patterns in roentgenograms. There is unquestionably an opportunity for important contributions in these areas, and the author draws upon his own deep interest and long experience. The book begins with an excellent description of the history and basic conceptions of the pneumoconioses. While the presentation of basic conceptions is generally good and includes a discussion of the shortcomings of the chemical and piezoelectric theories of silicosis, it ends at that point without mention of more recent theories and evidence on the surface chemistry of free silica particles and its possible role in the pathogenesis of silocisis.

Much good information is presented in the book concerning roentgenographic principles in silicosis and asbestosis. However, it is not well systematized and, frequently, a truly important notion pertinent to pneumoconiosis is buried among rather commonplace remarks on roentgenographic manifestations of disease processes having only an incidental connection with pneumoconiosis. For example, the book is replete with such statements as "the nodes may be densely calcified; irregularly calcified; or the calcium may be deposited in such a way as to produce an egg shell appearance." This does appear to cover all possibilities, but it is difficult to see what contribution it makes to a roentgenologist's conception of pneumoconiosis. If it is Dr. Pendergrass's opinion that the type of calcification has no diagnostic significance, it would seem that he might have said so and might have presented evidence to refute the widespread belief that "egg shell calcification" in the presence of pulmonary nodulation is highly suggestive, if not pathognomonic, of silicosis.

The above quotation is shortly followed by another which is italicized for emphasis "Such evidence (calcification of nodes) is not, as a rule, thought to be significant enough to justify recommending that the patient be removed from his particular job unless there are other modifying influences." It would have been helpful if Dr. Pendergrass had quoted, from his extensive experience, data concerning the number of persons with such calcification who have succeeded in working in silica dust for prolonged periods without reactivation of the infectious process. A number of other highly significant statements could have been enhanced by presentation of numerical data. Curiosity is also aroused but not satisfied as to what the other modifying influences might be.

Finally, it should be noted that the discussion emphasizes silicosis and asbestosis and includes brief comments concerning "coal worker's pneumoconiosis," but completely overlooks several other pneumoconioses such as Shaver's disease and the diseases occurring in the diatomaceous earth and beryllium industries.

The book includes a good bibliography of some 168 references, extending from Ramazzini and Zenker in the eighteenth and nineteenth centuries to late 1957.

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BOOKS RECEIVED

60 Jahre Medizinische Radiologie. Probleme und Empirie. By Hans R. Schinz. Pp. 275, Georg Thieme Verlag, Stuttgart, Germany (In U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $4.65.

Elementary Statistics, with Application in Medicine and the Biological Sciences. By Frederick E. Croxton. Pp. 376, Dover Publications, Inc., New York, 1959. Price, $1.95.

The Year Book of Medicine (1959–1960 Series). Edited by Paul B. Beeson, *et al.* Pp. 733, The Year Book Publishers, Inc., Chicago, Illinois, 1959. Price, $8.00.

Symposium on Pulmonary Ventilation. Edited by R. P. Harbord and R. Woolmer. Pp. 109, The Williams & Wilkins Co., Baltimore, Md. (U. S. agents), 1959. Price, $4.00.

L'Expertise de la Silicose Pulmonaire. By Paul Sadoul and Maurice Dusapin. Pp. 234, Masson & Cie., Paris, France, 1959.

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The Mediastinum. By Ted F. Leigh and H. Stephen Weens. Pp. 246, Charles C Thomas, Springfield, Illinois, 1959. Price, $11.50.

Die Streifenatelektasen der Lunge. By Friedrich Heuck. Pp. 108, Georg Thieme Verlag, Stuttgart, Germany (in U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $6.70.

Das Röntgenschichtbild. By A. Gebauer, *et al.* Pp. 454, Georg Thieme Verlag, Stuttgart, Germany (in U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $35.25.

Pediatric Pathology. By Daniel Stowens. Pp. 676, The Williams & Wilkins Company, Baltimore, Maryland, 1959. Price, $20.00.

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Homologous Serum Jaundice

A Statement of the Committee on Diagnostic Skin Testing

The tuberculin test is an essential tool for the detection of persons who have been infected with tubercle bacilli, and mass tuberculin testing will almost certainly play an important role in the ultimate eradication of tuberculosis.

over the past ten years, millions of intradermal tuberculin tests have been administered by federal and state agencies and others employing the multi-dose syringe. There is no clinical or epidemiologic evidence that homologous serum jaundice or other infections have occurred with any greater frequency in those who received tuberculin tests than in those who did not. In one medical school, approximately 900 medical students received more than 5,000 skin tests from multi-dose syringes over a twelve-year period. These students were under close medical supervision for two years following the injections, and not one case of homologous serum jaundice developed. In another large university, tuberculin tests are given to an average of 7,500 students each year. Over the last ten years only one case of hepatitis was seen; and that was in a medical student who had been in contact with patients with infectious hepatitis. At this institution, every absence from class due to illness comes to the attention of the same staff members who administer the tuberculin test.

In the process of giving the tuberculin test, the outside of the needle does become contaminated with tissue juices. Therefore, in intradermal testing a sterilized needle should be used for each test, or the needle should be sterilized between tests by heating the needle to redness in a living flame.

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NOTICES

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15-20
1961—Cincinnati, Ohio—May 21-26
1962—Miami Beach, Florida—May 20-25
1963—Denver, Colorado—May 12-17
1964—New York, New York—May 24-29

THE AMERICAN REVIEW
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ABSTRACTS

Edited by EDWIN D. KILBOURNE

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or pulmonary diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 207 journals which represent 31 countries and which are published in 18 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Differential Pulmonary Artery Occlusion in Patients With Chronic Pulmonary Disease. B. L. Charms,* B. L. Brofman, and A. Adicoff. *Am. J. Med.*, April, 1959, 26: 527–533. (*Mt. Sinai Hosp., Cleveland, O.)

Hemodynamic studies are reported in 5 patients with pulmonary disease, both localized and generalized, in whom first one main branch of the pulmonary artery and then the other were occluded. The method of temporary unilateral pulmonary artery occlusion has proved to be a useful adjunct in evaluation of patients with pulmonary lesions, and especially in determining pulmonary resectability when diffuse disease is also present.

T. H. Noehren

Pulmonary Artery Stenosis. H. A. Shafter* and H. A. Bliss. *Am. J. Med.*, April, 1959, 26: 517–526. (*U. of Illinois College of Med., Chicago)

This report adds 5 patients with pulmonary artery stenosis to the 28 previously described. The clinical diagnosis of pulmonary artery stenosis is frequently hampered by coexistent cardiovascular lesions. A continuous murmur should make one think of the diagnosis. The lesion more commonly produces a systolic murmur which may be heard with maximal intensity at the base of the heart several centimeters lateral to the sternal border. Chest roentgenograms and fluoroscopy may show evidence of right ventricular enlargement. The main pulmonary artery segment may be normal or prominent, but a central narrowing of the hilar vessels has been noted in some patients with pulmonary artery stenosis. Pulmonary vascularity may be increased, decreased, or normal, depending on the hemodynamic effects of the stenosis and associated lesions.

The diagnosis of stenosis of the main branch of the pulmonary artery can be made at the time of catheterization of the right side of the heart by the observation of an abrupt pressure rise as the catheter is withdrawn through the narrowed segment. The significance of the lesion depends on the resistance it offers to right ventricular outflow. The narrowing which causes a small pressure gradient at rest may, with exercise, produce significant right ventricular hypertension. In most patients, pulmonary artery stenosis does not cause serious hemodynamic abnormalities. If these are present, however, surgical repair may be possible and desirable.

T. H. Noehren

Trigger Mechanisms in Asthma. R. H. Overholt.* *Dis. Chest*, June, 1959, 35: 587–596. (*Overholt Clinic, Boston, Mass.)

Although asthma is considered a medical disease, irreversible structural changes in the bronchial system may exist. In the past, the attention of most surgeons centered on interventions which interrupted the efferent or afferent nerve pathways to the lung. Structural abnormalities were not hunted for and corrected. Overlooked "triggers" quite possibly contributed to the disappointing lasting results of denervation alone. It has lately been recognized that disturbances in one area of the bronchial system may act as a "trigger" to set off general bronchospasm.

Intractable cases of asthma should have the benefit of a thorough investigation. At times, subjective or objective signs are present which attract attention to one side or the other. With or without bronchographic indication of abnormality, there may be strong presumptive evidence of mechanical difficulty which may be discovered at exploration and can be corrected. The surgeon inspects and overhauls the defective or abnormally functioning ventilatory organ. He liberates abnormally anchored segments, divides adhesions or bands, decorticates constricted segments, removes enlarged nodes or destroyed segments, denervates a spastic lung, and works with it until all healthy segments are working properly.

Long-term results (18 months to 12 years) have shown all 31 patients to be improved to some degree, with one-half completely free of asthma and 10 requiring occasional medication.

E. A. Rouff

Injuries to Main-Stem Bronchi: Complete Separation of Left with Successful Anastomosis and Instability of Right Due to Fracture of Multiple Cartilages. J. D. Hardy.* *Ann. Surg.*, June, 1959, 149: 949–959. (*U. of Mississippi Med. Center, Jackson)

Injuries to the main-stem bronchi are increasing in frequency, and are almost always the result of closed chest trauma, usually from automobile accidents. Pneumothorax, subcutaneous or mediastinal emphysema, hemoptysis, and evidence of cardiopulmonary embarrassment, often associated with rib fractures, should prompt one to consider the possibility of bronchial injury.

Two patients who sustained multiple injuries in automobile accidents are reported. The first patient had complete separation of the left main-stem bronchus, and continuity and lung expansion were restored at thoracotomy 11 weeks following injury. The second patient had fractures of several

adjacent cartilages in the right main-stem bronchus. The right lung could not be expanded until the kink in the collapsed bronchus had been corrected at surgery.

Following complete separation and occlusion of the bronchus, mucus continues to be secreted into the distal bronchus until the appearance of bronchiectasis is produced. This picture may be reversed after bronchial patency is restored and the lungs cleared of the copious, viscid, but often sterile, secretion.

M. J. SMALL

Nonspecific Bronchitis (in Finnish). O. M. PENTTI.* *Duodecim*, April, 1959, 75: 301–309. (*U. of Helsinki, Finland)

A nonspecific inflammatory process in the bronchial wall had caused in an asthmatic patient a stenosis leading to complete atelectasis of the upper lobe of the left lung. The diagnosis of nonspecific chronic bronchitis was made microscopically, after the left upper lobe was removed due to suspected malignancy.

J. HAAPANEN

The Rational Approach in the Use of Bronchodilators in Chronic Respiratory Disease. S. M. FARBER* and R. H. L. WILSON. *Ann. Int. Med.*, May, 1959, 50: 1241–1251. (*U. of California School of Med., San Francisco)

The mechanisms for the obstructive wheezing found in asthma, chronic bronchitis, senile emphysema, and bullous emphysema are discussed. Bronchospasm is a variable part of the mechanism of airway obstruction but is almost never the whole cause. In the majority of cases it is not an important cause. Therefore, the use of nebulized sympatheticomimetic agents to reduce bronchial mucosal edema as well as relax bronchial muscle, together with basal bronchial muscle relaxation by such substances as ephedrine, appear to offer the most help. Parasympatheticomimetic agents are discussed and their occasional usefulness by nebulization is pointed out; however, it is believed that they belong to the group of drugs best administered systemically. The usefulness and the limitations of aminophylline and other similar agents are considered both in increasing respiration and improving cough, and in providing relaxation of the bronchial musculature. It is realized that the best results with aminophylline will be obtained in the patient in whom bronchospasm and secretions are the major problems.

The direct bronchodilators form only a part of the whole treatment of the patient with obstructive emphysema causing wheezing. Yet these substances probably form the backbone of treatment of the average case and the best mechanism of using them will provide a better therapeutic result with less systemic toxicity (Authors' summary).

T. H. NOEHREN

Pruritus and Purpura as Unusual Symptoms of Carcinoma of the Bronchus. J. GOLDSTEIN* and J. HART. *Tubercle*, April, 1959, 40: 119–120. (*Joyce Green Hosp., Dartford, England)

For the past 20 years, carcinoma of the bronchus has been increasing in frequency. Its clinical diagnosis without the aid of roentgenographic examination, however, has not become any easier, and the disease occasionally masquerades in the guise of other conditions, such as arthropathy, diseases of the nervous system, and of the skeletal muscles. To the existing list of its symptoms are added two others which were the first features in a recent case, namely, pruritus and purpura.

The occurrence of thrombocytopenic purpura in cases of cancer has been mentioned occasionally, but pruritus has not, except in lymphadenoma, leukemia, and other reticuloses. Pruritus, as a rarity, has been observed in types of cancer other than that of the bronchus, as, for instance, in cancer of the stomach. In the case now described, the primary roentgenogram of the chest bore all of the characteristics of lymphadenoma, and the post-mortem diagnosis of cancer of the bronchus came as somewhat of a surprise.

It would be interesting to investigate the pruritus more fully in any subsequent case displaying similar symptoms in the light of recent studies indicating the role of various proteinases in its production. This case indicates that the first symptom of a carcinoma of the bronchus may be intractable pruritus, and that this should therefore be considered one of the rarer symptoms of the condition.

M. J. SMALL

Idiopathic Cardiac Hypertrophy With Endomyocardial Fibrosis: Report of a Case Developing after Recovery from a Purulent Lung Abscess. P. MCNAMARA,* W. JACOBS, and R. JAFFE. *Ann. Int. Med.*, April, 1959, 50: 1035–1045. (*Kings County Hosp., Brooklyn, N. Y.)

This case report is an example of idiopathic cardiac hypertrophy. It is of interest because it is considered the first case report of a patient initially seen with no abnormal cardiovascular manifestations, only to be followed subsequently for a period of 33 months during which the patient suffered from congestive heart failure until he

died from myocardial failure, recurrent pulmonary infarctions, and secondary cor pulmonale.

T. H. NOEHREN

Pulmonary Arteriovenous Fistula without Signs of Anoxemia: Report of a Case and Review of the Literature (in Japanese). A. TAKEUCHI,* S. ISE, T. MORIOKA, and T. HIRAI. *Jap. J. Thoracic Surg.*, July, 1959, 121: 557–559. (*Osaka U. School of Med., Japan)

A case report is presented of an 18-year-old male patient whose chief complaints were hemoptysis of several days' duration and generalized malaise. A roentgenogram and tomographs revealed a thumb-sized, oval, tuberculoma-like shadow in the left middle lung field. Surgery was performed with a preoperative diagnosis of pulmonary neoplasm, which turned out to be an arteriovenous fistula in the lingular portion of the left lung. Convalescence was uneventful and review of the 170 reported cases of pulmonary arteriovenous fistula gave the authors an impression that fatal complications due to hemorrhage may occur suddenly in this disease, as signs and symptoms of anoxemia due to arteriovenous shunt are absent and, therefore, that this disease should be called pulmonary arteriovenous aneurysm rather than fistula. (Hemoptysis or bloody sputum was present in 85 cases, absent in 17 cases, and no mention was made in 68 cases.)

I. TATENO

Asian Influenza A in Boston, 1957–8. I. Observations in 32 Influenza-Associated Fatal Cases. C. M. MARTIN, C. M. KUNIN, L. S. GOTTLIEB, M. W. BARNES, C. LIU, and M. FINLAND.* *A.M.A. Arch. Int. Med.*, April, 1959, 103: 515–531. (*Boston City Hosp., Mass.)

Studies of 32 Asian influenza A-associated fatalities during 1957–1958 in Boston and its environs reveal a spectrum of clinical, laboratory, and postmortem findings not unlike those associated with fatal cases during earlier influenza epidemics. Strains of Asian influenza A virus were isolated from tissues of 44 per cent of the cases, and influenza A viral antigen was demonstrated in interstitial macrophages of respiratory tract tissues in 31 per cent. Except when Gram-stain sputum studies revealed specific pathogens or when roentgenograms demonstrated pulmonary cavitation, it was not possible to make prompt diagnostic differentiation between pure influenzal pneumonia and postinfluenzal bacterial pneumonia. It is suggested that, in times of epidemic influenza and in areas where staphylococcal infections are highly prevalent, cases presenting signs usually associated with fatal influenza should be treated promptly as bacterial—particularly staphylococcal—pneumonias, unless or until that diagnosis is disproved. The need for a practical "artifical lung" is emphasized.

W. J. STEININGER

Asian Influenza A in Boston, 1957–8. II. Severe Staphylococcal Pneumonia Complicating Influenza. C. M. MARTIN, C. M. KUNIN, L. S. GOTTLIEB, and M. FINLAND.* *A.M.A. Arch. Int. Med.*, April, 1959, 103: 532–542. (*Boston City Hosp., Mass.)

The clinical and laboratory findings in 11 fatal and 9 nonfatal cases of staphylococcal pneumonia complicating Asian influenza A in the Boston area during 1957–1958 are compared. The fatal cases were generally marked by more severe and rapidly progressing illness, less accurate diagnosis, and relatively ineffective antimicrobial therapy. Although most of the 20 cases arose in the community rather than in hospital, the strains of staphylococci responsible were commonly resistant to multiple antimicrobials and were of bacteriophage types most often associated with hospital-acquired infections. Disk-sensitivity tests afforded a rapid, relatively accurate guide for the selection of effective drugs. It is suggested that, during epidemic influenza in areas where major staphylococcal infections are common, all patients with signs of potentially fatal influenza be diagnosed and treated promptly as cases of staphylococcal pneumonia caused by the prevalent types of antimicrobial-resistant staphylococci until the diagnosis can be disproved.

W. J. STEININGER

Virus Isolation in Fatal Human Cases of Asian Influenza: Comments on Current Literature. R. J. BLATTNER. *J. Pediat.*, July, 1959, 55: 113–115.

Recent literature on this subject is reviewed with the conclusion that fulminant influenzal pneumonitis without superimposed bacterial infection does occur, although the importance of secondary bacterial invasion is well established, notably by hemolytic staphylococci, *Hemophilus influenzae*, and pneumococci. Treatment of these fulminating cases poses a serious problem since the course of the disease is so rapidly fatal that there is little opportunity for effective therapy. The general use of influenza vaccine is suggested as the only available means of decreasing this type of mortality. On the basis of these clinicopathologic studies and of those concerned with virus isolation, the investigations lend support to

the concept of viremia in overwhelming influenzal infection in man.

M. J. SMALL

Serologic Response to Far East Influenza. I. GRESSER* and S. B. HALSTEAD. *A. M. A. Arch. Int. Med.*, April, 1959, 103: 590–592. (*406th Med. General Lab., San Francisco, Calif.)

The hemagglutination-inhibition (HI) and complement-fixation (CF) antibody response in military personnel to "Far East" influenza virus is described. The CF test was of value in the early diagnosis of epidemic influenza, a rise in titer occurring by the second week of illness. HI antibody appeared at maximal values after the third week of illness. This response is at variance with previous observations which showed that CF, HI, and neutralizing antibodies to influenza develop early and in parallel maximal titers.

W. J. STEININGER

Kartagener's Triad: A Family Study. G. W. GORHAM* and J. G. MERSELIS. *Bull. Johns Hopkins Hosp.*, January, 1959, 104: 11–16. (*Johns Hopkins Hosp., Baltimore, Md.)

The case histories of two siblings with Kartagener's triad (coexisting bronchiectasis, situs inversus, and sinusitis) are presented, together with a detailed study of their family tree and a review of 38 similar cases reported since 1950. The facts reported show that Kartagener's triad has a high familial incidence but appears only in one generation. It is suggested that an environmental factor during embryonic development, rather than a genetic factor, may account for the appearance of the syndrome.

C. H. PIERCE

Spontaneous Disappearance of Bilateral Pulmonary Metastases. Report of a Case of Adenocarcinoma of Kidney after Nephrectomy. L. KESSEL.* *J. A. M. A.*, April 11, 1959, 169: 1737–1739. (*State Veterans Hosp., Rocky Hill, Conn.)

A 65-year-old man was found by roentgenographic examination to have pulmonary lesions suggesting metastases of a tumor. Intravenous pyelography gave evidence of a kidney tumor. A nephrectomy was performed and a papillary adenocarcinoma was removed. During the first 2 months after operation, the metastases increased in size and number. A chest roentgenogram, made 4 months after operation, however, showed complete clearing of both lung fields. On follow-up examination 7 months later, both lung fields were free from demonstrable metastases.

H. ABELES

Cavitary Lesions in Sarcoidosis. K. A. HARDEN* and A. BARTHAKUR. *Dis. Chest*, June, 1959, 35: 607–614. (*Howard U., Washington, D. C.)

Three proved cases are presented of sarcoidosis which was uncomplicated by tuberculosis and which exhibited rarefactions on chest films. Autopsy and exploratory thoracotomy confirmed the diagnosis in each instance. It is believed that there are at least three types of pulmonary lesions occurring in sarcoidosis which appear as rarefactions on the roentgenogram: (*1*) true cavitary lesions, (*2*) emphysematous bullae and blebs, and (*3*) ectatic bronchi and bronchiectasis.

E. A. ROUFF

Cystic Lung Lesions From Metastatic Sarcoma. N. CROW* and B. BROGDON. *Am. J. Roentgenol.*, February, 1959, 81: 303–304. (*USAF Hosp., Parks Air Force Base, Calif.)

A case is reported of metastatic sarcoma of the lungs which presented as thin-walled cysts. This case is of interest because of the initial cystic manifestation of pulmonary metastases. Subsequently, apparent tumor developed. The more common cycle of tumor, necrosis, and then cavity formation did not become evident until late in the course of the disease. It is believed that this case may further explain the 5 reported cases of "spontaneous" pneumothorax associated with metastatic sarcoma.

T. H. NOEHREN

Eosinophilic Pneumonopathy (Loeffler's Syndrome): Case Report Describing Two Attacks in the Same Patient and Contrasting Steroid with Nonsteroid Therapy. B. P. SANDLER* and J. W. MAHONEY. *Am. J. M. Sc.*, May, 1959, 237: 624–630. (*V. A. Hosp., Oteen, N. C.)

Two attacks of eosinophilic pneumonopathy in the same patient, one treated with steroid and one with nonsteroid therapy, were so similar that they were comparable in the evaluation of the therapeutic effect of hydrocortisone on the syndrome. The prompt effect of the hormone on the eosinophilia, fever, subjective symptoms, and pulmonary infiltrations warrants the conclusion that the hormone had a powerful and beneficial effect on the etiologic factor. That the effect was temporarily suppressive was evident from the presence of small nodular lesions in both upper lung fields, without clinical symptoms, 47 days after cessation of therapy. Persistence of eosinophilia was also an indication that the fundamental factor was still operating.

W. J. STEININGER

Paragonimiasis of the Lung (in German). H. G. THIELE.* *Deutsche med. Wchnschr.*, April 10, 1959, 84: 752–756. (*Bernhard-Nocht-Institut, Hamburg 4, Germany)

The trematode *Paragonimus westermani* may infest the human lung as part of a complex life cycle. The case is reported of a Chinese seaman admitted to a hospital in Hamburg with the original diagnosis of, and treatment for, tuberculosis. In addition to the pulmonary shadows suggestive of tuberculosis, there was marked eosinophilia up to 27 per cent. The sputum contained eosinophils and Charcot-Leyden crystals. The diagnosis of paragonimiasis was made by finding typical worm eggs on microscopic examination of unstained sputum. In the "closed" form, i.e., without eggs in the sputum, the diagnosis can be made by means of a complement-fixation test or an intracutaneous injection of antigen. The latter method is not absolutely specific. In the described case, the treatment consisted of high doses of chloroquine orally, intramuscularly, and by aerosol. Despite these high doses, there were no untoward side effects.

J. HAAPANEN

The Syndrome of Chronic Pulmonary Disease and Gastroduodenal Ulceration. A Clinical Study and Literature Review. W. O. WEST,* R. O. BURNS, J. M. DANIEL, and H. A. JACKSON. *A. M. A. Arch. Int. Med.*, June, 1959, 103: 897–901. (*Ohio State U., Columbus)

Review of the records of a random group of 845 general medical patients showed a statistically significant increase of 27 per cent in the incidence of gastroduodenal ulceration in patients with chronic pulmonary disease. The etiology and pathogenesis are discussed. The majority of these patients were men younger than 40. Ulcer symptoms and complications were similar in these patients and in patients with gastrointestinal ulceration alone. The severity of the pulmonary disease did not correlate with the incidence of chronic gastroduodenal ulceration. It is concluded that every patient with chronic pulmonary disease should have gastroduodenal roentgenographic studies and examination of the stool for occult blood. If a patient with chronic pulmonary disease is anemic, a bleeding peptic ulcer should be suspected.

W. J. STEININGER

Pulmonary Infarction After Dental Extraction, Report of Two Cases with Recovery. C. W. ADAMS* and J. M. HUDGINS. *J. A. M. A.*, May 23, 1959, 170: 412–416. (*Vanderbilt U., Nashville, Tenn.)

Two case histories involving men aged 40 and 38 years, respectively, illustrate the occurrence of pulmonary infarction after extraction of teeth. One case commenced 5 days, and the other one 7 days, after tooth extraction. Both patients had cervical phlebothrombosis which led to the pulmonary complications.

H. ABELES

Three Endemic Outbreaks of Transient Pulmonary Infiltrates with Blood Eosinophilia (Löffler's Syndrome) (in German). C. HENGGELER.* *Schweiz. med. Wchnschr.*, April 11, 1959, 89: 402–404. (*Bahnhofstr. 7, Schwyz, Switzerland)

Three outbreaks of transient pulmonary infiltrates with blood eosinophilia are described. All 3 occurred during the summer months of June and July. This illness is generally known by the rather vague names of "Sommergrippe" or "influenza" and is usually not taken seriously. Sometimes, however, painful symptoms make it necessary to consult the doctor.

During the first epidemic, 8 infiltrates were observed, 1 in a boy who fell ill 4 times in 5 years. Besides these roentgenographic findings, some suspicious symptoms were observed in other persons of the same family, such as increased blood eosinophilia, coughing, and dyspnea—a total of 7 cases. The ascaris etiology of the illness was proved by the presence of ascaris eggs in the garden soil.

The second and third epidemics occurred during the years 1951 and 1958 in another family. The extraordinary intensity of the subjective symptoms and the marked objective findings were surprising features of the epidemic of 1951. The epidemic of 1958 was less severe. It is considered that quantitative factors at the time of the infection with ascaris larvae may determine the intensity of the disease.

J. HAAPANEN

Familial Pulmonary Alveolar Microlithiasis: Four Cases from Colombia, S. A. G. E. GOMEZ,* E. LICHTEMBERGER, A. SANTAMARIA, L. CARVAJAL, B. JIMENEZ-PENUELA, E. SAAIBI, A. R. BARRERA, E. ORDUZ C., and A. CORREA-HENAO. *Radiology*, April, 1959, 72: 550–561. (*Bogota, Colombia, S. A.)

Two features of pulmonary alveolar microlithiasis, namely, a characteristic roentgenogram showing the presence of innumerable minute intra-alveolar concrements, like grains of sand, most marked at the bases, and the complete absence of symptoms, make possible a roentgenographic diagnosis even without a confirmatory pathologic report. Death occurs as a result of cardiac insufficiency, due in turn to chronic cor pulmonale developing after 10, 20, or more years of apparently good health. The 4 cases here re-

ported, occurring in 4 sisters, are the first discovered in Colombia. It is suggested that microlithiasis may not be exclusively familial, but that environmental and dietetic factors may play a role in its onset.

W. J. STEININGER

Thromboembolism of the Lungs. J. ARENDT* and M. ROSENBERG. *Am. J. Roentgenol.*, February, 1959, 81: 245–254. (*Chicago Med. School, Ill.)

Thrombo-embolism without infarction presents as an abnormal transparency of the involved lung segment with absence of one or both of the large hilar vessels. The heart shadow assumes a peculiar shape, best described as the "amphoric" type. In the absence of both hilar vessels (pulmonary arteries), a large saddle embolus must be considered.

A thrombus or embolus which is located slightly more peripherally within the main branches of the pulmonary arteries causes a buckling and aneurysmal widening of the vessel, with either a sharp cut-off or tapering at the peripheral end of the embolus. Such thrombus or embolus is compatible with life, and leads to the development of either acute or chronic cor pulmonale. In contrast to the cor pulmonale of obstructive emphysema, the position of the diaphragm is elevated, the heart does not assume the vertical position, and within a short time develops enlargement of inflow and outflow tracts of the right ventricle.

Thrombo-embolism with infarction does not, as a rule, present Westermark's sign of increased translucency of a lung segment, since it occurs mostly in a congested lung with dilated bronchial arteries and numerous collaterals. Infarction is common in mitral stenosis, hypertensive heart failure, and coronary insufficiency. Infarcts represent an area of ischemic necrosis usually located at the periphery of the lung. They are rarely "wedge-shaped," as the apex of the wedge is cut off. A "truncated cone" or an "umbrella" shape results which is quite characteristic. Smaller infarcts frequently have a cuboid or meniscus-like appearance. In the costophrenic angle, the "hump-shaped" elevation on the medial side of a small pleurisy has frequently been observed and appears diagnostic. The base of the embolic infarcts is sometimes the interlobar fissure line, along which they form a parabolic shadow, which is best seen on a lateral roentgenogram.

T. H. NOEHREN

Staphylococcal Pneumonia in Children and Adults. H. I. MEYERS* and G. JACOBSON. *Radiology*, May, 1959, 72: 665–671. (*Los Angeles County Hosp., Calif.)

Staphylococcal pneumonia has become an increasing problem in children, particularly in infants under 6 months of age, in whom the largest number of cases occur and in whom the mortality rate is over 50 per cent. The roentgenographic picture is usually characteristic enough to suggest an exact etiologic diagnosis early in the disease, often before bacteriologic studies are available. In infants and small children, characteristic roentgen features are those of rapid change, pleural effusion or empyema, pneumatocele formation, and pyopneumothorax of variable extent. In the older age group, infiltration and lung abscess formation are more prominent. Treatment, often prolonged, depends upon isolation of the organism and administration of the correct antimicrobial.

W. J. STEININGER

Staphylococcal Septicemia With Recurrent Spontaneous Pneumothorax. A. SCHWEICH* and J. FIERSTEIN. *Ann. Int. Med.*, March, 1959, 50: 819–828. (*Montefiore Hosp., N. Y., N. Y.)

A case of staphylococcal septicemia is reported because it demonstrates a number of clinically significant features, particularly the development of recurring, spontaneous pneumothorax. It is believed that pneumothorax may be the result of either necrotizing pulmonary lesions or the development of postinfectious pneumatoceles or subpleural blebs.

T. H. NOEHREN

Studies of the Pathogenesis of Thoracic Deformities (in German). M. MANEKE.* *Deutsche med. Wchnschr.*, March 13, 1959, 84: 504–509. (*Gesundheitsamt, Hannover, Germany)

Follow-up examination, analysis of case notes, biochemical tests (inorganic phosphates, calcium and alkaline phosphatase), and roentgenographic examinations of 90 children with deformities of the bony thorax (funnel chest, pigeon chest, "bell-shaped" chest, or Harrison's groove), indicated that rickets could be excluded as a cause of such deformities. Study of the children's blood relations revealed in all instances a hereditary disorder of fibroblasts which took different forms in the various generations. Funnel and pigeon chests are thus seen only as extreme variants of one and the same sternocostal "dysostosis," which can assume a variety of expressions and *formes frustes*. Rickets may, as an additional or incidental disease, act as a precipitating factor or make the deformity manifest prematurely, but it does not play a role in the pathogenesis of the above deformities. The prophylactic and therapeutic consequences of these findings are discussed.

J. HAAPANEN

Primary Tumors and Cysts of Mediastinum. T. H. Wan,* C. Y. Jen, and M. H. Shih. *Chinese M. J.*, May, 1959, 78: 424–433. (*Shanghai First Med. College, China)

Sixty-two cases of primary mediastinal tumors and cysts seen during the period of 10 years ending March, 1957, are reviewed. This low number of cases indicates the relative rarity of primary mediastinal tumors. There were 22 cases of neurogenic tumor, 12 of thymomas, 10 of lymphosarcoma, 10 of teratoid tumor, 2 of bronchogenic cysts, and 6 cases with various other types of tumors. Excluded from this study were: (*a*) undifferentiated carcinoma or carcinoma of undetermined type apparently arising from the thymus, but without a thorough autopsy study; (*b*) tumors of the bony thoracic cage protruding into the mediastinum; (*c*) muscle wall tumors of the esophagus; (*d*) granulomatous lesions of the mediastinum, such as tuberculoma; and (*e*) leukemias and Hodgkin's disease. The clinical and roentgenographic characteristics of these tumors are reviewed.

The tumors were surgically excised in 44 patients, and there was one postoperative death. Early surgical intervention is advised except for patients with lymphoma.

L. Hyde

Primary Malignant Tumors of the Lung Other than Bronchogenic Carcinoma. G. V. Brindley, Jr.* *Ann. Surg.*, June, 1959, 149: 936–948. (*Scott and White Clinic, Temple, Tex.)

Most primary malignant tumors of the lung are bronchogenic carcinomas but, occasionally, other types occur. It is essential that these lesions be recognized and the most effective treatment be administered promptly to assure the patient his best prognosis. The diagnosis and management of 10 patients with alveolar cell carcinoma of the lung, 2 patients with primary pulmonary hemangioendothelioma, and other patients with lymphosarcoma, fibrosarcoma, or neurofibrosarcoma are reviewed. Preferable forms of therapy are suggested (Author's summary).

M. J. Small

Eosinophilic Granuloma of the Lung. Clinical Features and Connective Tissue Patterns. A. E. Anderson, Jr.,* and A. G. Foraker. *A. M. A. Arch. Int. Med.*, June, 1959, 103: 966–973. (*Baptist Memorial Hosp., Jacksonville, Fla.)

Two cases of eosinophilic granuloma of the lung, proved by lung biopsy, showed some variation from previous descriptions and interesting connective tissue alterations. Both patients were severely disabled with respiratory insufficiency, and appeared terminal. Cortisone therapy produced striking symptomatic relief. Microscopically, "early," more productive lesions were associated with reticulin fibrosis. With progression of the sclerosing process, there was an apparent conversion of reticulin to collagen. Disruption and destruction of the elastica were pronounced and widespread in both cases.

W. J. Steininger

Pulmonary Histiocytosis X (Eosinophilic Granuloma). J. Pagan-Carlo* and T. Haley. *Am. J. Roentgenol.*, February, 1959, 81: 231–235. (*Touro Infirmary, New Orleans, La.)

The problem of the so-called eosinophilic granuloma, apparently primary in the lung, is reviewed. The variation of protean manifestations of this pathologic entity, labeled as histiocytosis X, poses the question whether there is such a disease as primary eosinophilic granuloma of the lung. It is the purpose of this paper to emphasize the roentgenographic manifestations of this disease in the lungs, and at the same time to stimulate consideration of the possibility that the reported cases of primary eosinophilic granuloma of the lung (pulmonary histiocytosis X) may simply represent the "monosymptomatic expression of a disseminated process."

Some cases have cleared up spontaneously, others have been greatly benefited by roentgen therapy or by steroids. The success of therapy depends on an early diagnosis and the stage or activity of the process, together with the presence or absence of long-term involvement of vital organs. When the disease is in the active proliferative stage, the use of steroids is indicated; in the later stages of the disease, with extensive pulmonary fibrosis and severe emphysema, direct therapy probably will be of little, other than supportive, value.

T. H. Noehren

The Vanishing Lung Tumor—Pulmonary Hematoma. J. Williams.* *Am. J. Roentgenol.*, February, 1959, 81: 296–302. (*U. S. Naval Hosp., St. Albans, N. Y.)

Four cases of hematomas following nonpenetrating chest trauma are presented and the differentiation of these tumefactions from "coin lesions" are discussed. Roentgenographically, pulmonary hematomas may present as single or multiple spherical densities, usually measuring a few centimeters in diameter, or as a spindle-shaped density occurring within the lung parenchyma. Changes of pulmonary contusion and rib fractures may be present. The only sign of roentgenographic

differentiation from other "coin lesions" is the central area of radiolucency which, at times, occurs within the hematoma, and the postoperative hematomas. This sign is observed in the early stage of the formation of the hematoma. Later, there appear to be no differentiating factors from other pulmonary tumefactions; therefore, the history is of prime importance.

T. H. NOEHREN

TUBERCULOSIS, PULMONARY

The Course of Active Tuberculosis Complicated by Pregnancy. P. FLANAGAN* and N. M. HENSLER. *J. A. M. A.*, June 13, 1959, 170: 783–787. (*U. S. A. F. Hosp., Parks Air Force Base, Calif.)

The course of tuberculosis was observed in a group of 22 pregnant women and compared with that in a group of 40 nonpregnant women of the same average age. There was no evidence that pregnancy affected the prognosis of tuberculosis provided the disease was treated similarly in both groups.

H. ABELES

The Ghon Focus of Tuberculous Reinfection. L. ARANY.* *Am. J. Roentgenol.*, February, 1959, 81: 207–210. (*V. A., Roanoke, Va.)

The typical roentgenographic appearance of the active primary tuberculous complex is a dumbbell shadow, one end of which is the parenchymal focus and the other the lymph node component. In due course of time, both shadows become smaller, denser, and more sharply delineated, and finally undergo calcification and ossification. The size of these round calcific deposits is usually 0.5 cm. or less in diameter.

Anatomic study on innumerable cases seems to establish the fact that a primary complex is structurally characteristic and pathognomonic for the primary infection. Until 1940, it was generally accepted that any subsequent infection with tubercle bacilli would result in the reinfection type of reaction, and a second primary complex would never be formed. In that year, Terplan reported on 29 autopsies in which formation of typical complexes due to reinfection was found after complete healing of the real primary complex. Apparently, in these cases the body, in spite of a prior infection, returned to the same type of reactivity that existed before the first infection had taken place.

The case is presented of a tuberculin-positive and histoplasmin- and coccidioidin-negative individual in whom the hilar component of the first infection and the parenchymal component of the reinfection assumed the roentgenographic appearance of a bipolar primary complex. Waning of a previously present tuberculin sensitivity is not a necessary prerequisite to formation of a Ghon focus of reinfection.

T. H. NOEHREN

TUBERCULOSIS, NONPULMONARY

Tuberculoma of the Brain with Tuberculous Adenitis and Epididymitis. S. R. COGAN* and J. S. BORNSTEIN. *Ann. Int. Med.*, March, 1959, 50: 796–804. (*Michael Reese Hosp., Chicago, Ill.)

The incidence of tuberculoma of the central nervous system has been decreasing markedly over the last half century, in keeping with the decrease observed in all types of tuberculosis. Autopsy statistics have shown this decrease to be from 50 per cent at the turn of the century to the present 3 to 5 per cent expressed as the percentage of all brain tumors in large case series.

Most intracranial tuberculomas do not give rise to symptoms—they are found at necropsy. Symptomatic cases present an expanding intracranial lesion, with or without focal neurologic signs, and are indistinguishable from brain tumor. Vague central nervous system symptoms may be present for a variable period of time before the onset of the above picture. Intracranial tuberculoma is part of a widespread heavy dissemination of tubercle bacilli occurring in the immediate or remote past. From the diagnostic standpoint, this understanding as to the pathogenesis of these lesions has a twofold significance. The first is that, in patients presenting with the clinical syndrome of a space-occupying intracranial lesion and a known site of extrapulmonary tuberculosis, the presence of intracranial tuberculoma will be far more common than in patients presenting with the cerebral picture and evidence of pulmonary tuberculosis. Evidence for this may be adduced by comparing the relatively low incidence of intracranial tuberculoma in cases of pulmonary tuberculosis with the high incidence of systemic tuberculosis in patients with cerebral lesions.

To prevent postoperative meningitis, as well as to treat occult sites of tuberculosis, long-term chemotherapy for at least 2 and probably 3 years should be started in these patients. Prior to the advent of streptomycin, the postoperative incidence of meningitis was practically 100 per cent, and it was uniformly fatal. This has dropped to an incidence of from 10 to 20 per cent with streptomycin, and the outlook for the future is even better with more recently developed chemotherapeutic agents, especially isoniazid.

Patients with intracranial tuberculoma should be periodically checked for development of other extrapulmonary lesions. A case, diagnosed preoperatively, of intracranial tuberculoma with tuberculous adenitis and epididymitis, is presented.

T. H. NOEHREN

Some Unusual Cases of Cerebral Tuberculosis in the Elderly. A. B. KING.* *Bull. Johns Hopkins Hosp.*, February, 1959, 104: 75–88. (*Guthrie Clinic, Sayre, Penn.)

Cerebral tuberculomas, although rare, are occasionally encountered in patients with known active tuberculous infection. Four case histories are presented, however, which demonstrate rapid fulminating tuberculous meningitis in elderly patients who had negative chest roentgenograms but who showed far advanced cerebral tuberculomas at autopsy. Due to the age group and lack of evidence of active tuberculosis, the cerebral symptoms were at first accounted for by factors other than tuberculomas. When the diagnosis of tuberculous infection was considered, vigorous treatment with antimicrobials was without avail. Although tuberculous meningitis is most commonly found in the younger age group, these findings point up the fact that the possibility of all forms of tuberculosis must be considered among geriatric patients so that early treatment can be instituted.

C. H. PIERCE

Results of Bacteriologic Examination of Menstrual Blood in Genital Tuberculosis (in German). E. KRÖGER* and H. KRÄUBIG. *Deutsche med. Wchnschr.*, May 1, 1959, 84: 859–865. (*Hygiene-Institut, Göttingen, Germany)

Bacteriologic examinations were made of 1,450 specimens of menstrual blood obtained from 716 patients. Of these, 82 (5.7 per cent) were positive for *M. tuberculosis*, representing 49 patients (6.8 per cent). Only 3 of these 49 had no history of genital tuberculosis. However, in 7 of these 49 patients, more than one bacteriologic examination was necessary before positive results were obtained.

It is suggested that in all suspected cases at least 3 specimens of menstrual blood should be examined bacteriologically, both by culture and animal inoculation. After the treatment, a control examination ought to be made. For the collection of menstrual blood, a portio pessary was used. In virgins, the specimens were obtained by repeated vaginal suctions. For cultures, solid egg or fluid serum media were considered the best.

J. HAAPANEN

THORACIC SURGERY

Contribution to the Healing of Bronchial Cancer (in German). K. JÓS.* *Ztschr. Tuberk.*, February, 1959, 112: 291–293. (*János Hospital, Budapest, Hungary)

The rate of definite healing was higher in 14 patients operated on for bronchial cancer who developed empyema postoperatively than in 14 others who did not have this complication. Therefore, an artificial empyema has been instituted with aleuronat suspension during the past three years after surgery for bronchial cancer.

J. HAAPANEN

Closed Chest Injury. J. H. FORSEE* and H. A. BLAKE. *A. M. A. Arch. Surg.*, May, 1959, 78: 791–793. (*Walter Reed Army Hosp., Washington, D. C.)

Severe intrathoracic injury may follow mild trauma to the chest wall. Certain features of cardiac injuries, which present a challenge and hope for successful surgical therapy, are discussed. Compression of the thoracic cage, pulmonary hematoma, traumatic lung cavitation, rupture of the tracheobronchial tree, and large-vessel injury with aneurysm formation and obstruction are briefly reviewed. It is believed that an immediate awareness of these and similar conditions will increase the acute as well as the late salvage rates following closed chest injury.

W. J. STEININGER

The Role of Surgery in the Management of Unilateral Pleural Effusion. T. FERGUSON* and T. BURFORD. *Ann. Int. Med.*, April, 1959, 50: 981–998. (*Washington U., St. Louis, Mo.)

Twenty-one cases of undiagnosed, persistent, unilateral, pleural effusion have been seen during the last 7 years. There were 16 males and 5 females in the series. The average patient age was 51. The known duration of fluid accumulation averaged 3½ months. Seven of the 21 effusions contained gross blood. After the usual diagnostic methods had failed, all of the patients had elective exploratory thoracotomy.

Only 3 patients proved to have malignant disease at operation. Of the remaining 18, 6 had tuberculous pleuritis and 12 had nonspecific inflammation of the pleura. The presence of blood in the effusion was of no help in determining whether the causative process was neoplastic or inflammatory. Of the 7 patients with bloody effusions, 1 had cancer and 6 had inflammatory disease.

All of the patients derived therapeutic benefit from operation. Restoration of lung function was

accomplished by decortication when a constricting membrane was present. Fluid re-accumulation was permanently abolished by the performance of a total parietal pleurectomy. The long-term follow-up has been quite satisfactory. Sixteen of the 21 patients are clinically well more than 3 years after operation, including 6 patients with active tuberculosis of the pleura.

Exploratory thoracotomy with pleural biopsy, decortication, and parietal pleurectomy should become an integral part of the management of every patient who has a persistent undiagnosed unilateral effusion (after authors' summary).

T. H. NOEHREN

Surgical Treatment of Hiatal Hernia: Indications, Technique, and Results (in German). E. KAISER.* *Schweiz. med. Wchnschr.*, May 16, 1959, 89: 526–529. (*Stadtspital Waid, Zürich, Switzerland)

Sixty-two cases of hiatal hernia were performed on 18 males and 42 females. Two females had surgery twice because of relapse. The operation was done only when conservative therapy had failed. A third of all of the cases were found to be associated with gallstones, which were removed at the same time. In spite of good results, the transthoracic method of Allison was abandoned because of a prolonged and complicated convalescence and lasting scar pains. Furthermore, this approach excludes cholecystectomy at the same time. The abdominal approach has proved successful, except in cases of enormous obesity. The narrowing suture of the pillars of the diaphragm in connection with gastropexy can be considered theoretically well founded and most successful in practice.

J. HAAPANEN

Bronchoscopic Investigations in 70 Cases of Sarcoidosis (in German). R. KÄMPFER.* *Tuberkulosearzt*, April, 1959, 13: 261–273. (*Selters-berg, Giessen, Germany)

Seventy cases of sarcoidosis are reported. Bronchoscopy was performed on 60 patients. In 19 cases the bronchial mucosa appeared to be normal. Biopsy was carried out 29 times with a positive finding for sarcoidosis in 19 instances, including some cases with negative bronchoscopic findings. The differential diagnosis between sarcoidosis and tuberculosis can sometimes be difficult by histologic examination of biopsy specimens of bronchial mucosa.

J. HAAPANEN

Surgery of Tuberculosis in Ceylon: A Review of Two Hundred and Fifty Cases. A. T. S. PAUL* and C. G. URAGODA. *Tubercle*, April, 1959, 40: 93–98. (*Thoracic Surgical Unit, General Hosp., Colombo, Ceylon)

This review covers the period from 1952–1956 during which a thoracic unit was first operating in Ceylon. It includes a group of chronic cases that could almost be called "permanent hospital residents" —those patients who had lived 5 to 10 years or more in sanatoriums. In many instances surgical treatment was carried out to relieve chronic disability caused by the persistent maintenance of artificial pneumothorax in unsuitable cases. Because of the different local conditions and temperament of the patients in Ceylon, it would be unfair to compare this report with similar reports from Western countries during the same period.

Of the 250 patients under review, 148 were followed up. This follow-up study, which was conducted in 1957, includes patients who were operated 1 to 5 years previously. Due to local conditions in Ceylon, the follow-up figures could not be improved. Seventy-eight of 126 patients in whom a resection was done were followed up: 28 were female, and 50 male: 62 were symptom-free. The mortality rate after pneumonectomy in this series was 23 per cent. Bronchopleural fistulas occurred in 14 per cent. There were 5 cases (of 126 resections) of postoperative hemorrhage, 1 of which was fatal.

Seventy of 115 patients who had thoracoplasties were followed up; of these, 23 were female and 47 male. Sixty-nine had improved since the operation and none that reported at the follow-up study had had positive sputum since thoracoplasty. Thirty-six patients still had symptoms, such as cough and expectoration or dyspnea, and 2 had a persistent sinus following infection of the Semb space. The safety of thoracoplasty in bad-risk cases was demonstrated.

M. J. SMALL

Pulmonary Resection for Metastatic Tumors. H. C. HABEIN, JR.,* O. T. CLAGETT, and J. R. MCDONALD. *A.M.A. Arch. Surg.*, May, 1959, 78: 716–723. (*Mayo Clinic, Rochester, Minn.)

Among 93 of 96 patients who had pulmonary resection for metastatic malignant lesions at the Mayo Clinic, 16 were living without evidence of recurrence 3 or more years after resection. Although 44 patients died less than 2 years after the metastatic pulmonary lesion was removed, 12 patients lived 2 or more years before succumbing. Another 6 patients were alive despite recurrent malignant tumors 2 or more years after resection.

Criteria for selection of patients for pulmonary resection for metastasis include adequate control of the primary lesion without evidence of local recurrence or other distant metastasis[5], and the presence of a single pulmonary lesion; if multiple, the lesions should appear as satellite nodules. The interval of time between resections of the primary tumor and the metastatic pulmonary growth is of prognostic significance. In this series there were no long-term survivors free of evident recurrence if the metastatic pulmonary tumor developed less than a year after the primary operation.

W. J. STEININGER

Intrathoracic Neurogenic Tumors in Infants and Children: A Study of Forty Cases. O. SCHWEISGUTH,* J. MATHEY, P. RENAULT, and J. P. BINET. *Ann. Surg.*, July, 1959, 150: 29–41. (*Hôp. Laennec, Paris, France)

A study of 40 cases of intrathoracic neurogenic tumors in children is presented: 15 neuroblastomas, 7 ganglioneuroblastomas, and 18 ganglioneuromas. Neurogenic tumor topography is classical: they are round, oval, or spindle-shaped, and are located posteriorly in the paravertebral gutter. The occurrence of intratumoral calcification in sympathetic tumor has been previously recorded. This was present in 10 of this series: 7 neuroblastomas and 3 ganglioneuroblastomas. Intratumoral calcification is a finding which favors immaturity or, at very least, a malignant tendency. Thoracic cage deformities appear related to tumor volume rather than growth characteristics.

Therapeutic indications are discussed, and surgery alone is advised for neuroblastomas in infants under 2 years of age and all ganglioneuromas. Postoperative X-ray therapy is the indicated treatment for ganglioneuroblastomas and neuroblastomas in children over 2 years of age. The results of treatment are given.

M. J. SMALL

PUBLIC HEALTH AND EPIDEMIOLOGY

Detecting Lung Cancer. R. D. YOUNG.* *Tubercle*, April, 1959, 40: 106–111. (*General Hosp., Bedford, England)

During the period 1947–1956, 291 patients were diagnosed as having lung cancers at a general hospital and chest clinic serving a population of approximately 160,000. The area concerned is the northern two-thirds of Bedfordshire which is served by a single general hospital with its attendant chest clinic. Sixty per cent were detected through chest clinic consultations, and 26 per cent through attending other departments of the hospital. Eight per cent were found through the "minifilm" sessions, to which general practitioners could refer their patients for chest films; 32 per cent of these patients had the tumors resected, compared with only 19 per cent of those detected through chest clinic consultations.

There was an average interval of 18½ weeks from the first recorded symptom to hospital attendance, with little change throughout the 10-year period. The interval from attendance to diagnosis was 5½ weeks; and it fell from 8 weeks in the first 5 years to 4 weeks in the second. From diagnosis to operation the average interval was about 3 weeks, making a total of 8½ weeks from the first attendance at hospital to surgical treatment.

The present review suggests that, at least in a rural area, the static "minifilm" camera, preferably situated at a chest clinic associated with the general outpatient department and wards of a general hospital, may be the most rewarding of the various means of detecting lung cancer. It seems particularly desirable to encourage general practitioners to refer for a "minifilm" every man past middle age recovering from anything but the mildest respiratory infection before he is allowed to return to work.

M. J. SMALL

An Investigation of the Validity of Death Certification of Cancer of the Lung in Leeds. G. M. BONSER* and G. M. THOMAS. *Brit. J. Cancer*, March, 1959, 13: 1–12. (*U. of Leeds, England)

Clinical records were traced on 999 death certificates recorded in Leeds in the years 1950–1954 which were coded as cancer of the trachea, pleura, lungs, or bronchi; 3.4 per cent were found to be incorrectly certified. The remainder were certified on unequivocal evidence (50 per cent), probable evidence (45 per cent), and doubtful evidence (5 per cent). The clinical notes of 879 similar cases recorded in the admission index of the 2 main hospitals in Leeds from 1950–1954 were compared with the death certificates. Of these, 92.5 per cent were correctly certified at death and, in 7.5 per cent, the presence of the disease for various reasons did not appear in the final coding. More cases were diagnosed clinically and not certified at death than were certified at death and not diagnosed clinically. Autopsy analysis showed that false positive certification was infrequent.

W. J. STEININGER

Transmission of the Common Cold to Volunteers Under Controlled Conditions. IV. Specific Immunity to the Common Cold. G. G. JACKSON* and H. F. DOWLING. *J. Clin. Invest.*, May, 1959, 38: 762–769. (*U. of Illinois, College of Med., Chicago)

It is concluded that the instillation of one of the five infectious secretions obtained from

patients with common colds gave specific protection against the development of common colds when the same secretion was instilled up to 45 weeks later, whereas there was no protection against colds produced by any other secretion. These observations suggest that the common cold is caused by a number of different viruses which are antigenically different and that, following infection, human subjects develop a specific immunity against the virus which lasts at least for several months.

E. DUNNER

Immunologic Evaluation of Monovalent Influenza Vaccine in Adults and in Children. H. B. BRUYN and E. H. LENNETTE.* *A.M.A. Arch. Int. Med.*, June, 1959, 103: 914–923. (*California State Dept. of Public Health, Berkeley)

The antibody response of young children and older adults to a monovalent Asian strain vaccine was compared, using subcutaneous and intradermal routes of administration with various amounts of vaccine. The extent of antibody response was found to be conditioned by prior infection with influenza A viruses or by poor immunologic exposure, through vaccination, to the antigens in the vaccine. In adults, a single injection of vaccine intradermally or subcutaneously produced a significant antibody response. In children, repeated injection of vaccine rather than increased antigenic mass was required to elicit significant antibody response. Whenever variation in influenza A virus results in the appearance of new strains with which the population has had little or no prior experience, repetition of inoculation appears to be as important in the adult as in the "immunologically inexperienced" child.

W. J. STEININGER

Epidemiologic and Clinical Observations in Sarcoidosis. M. CUMMINGS,* E. DUNNER, and J. WILLIAMS, JR. *Ann. Int. Med.*, April, 1959, 50: 879–890. (*V.A., Washington, D. C.)

Sarcoidosis was diagnosed in 1,700 patients seen in Veterans Administration hospitals between 1949 and 1956. The case records of 1,194 of these patients diagnosed during the 5-year period 1949–1954 were reviewed. Information concerning age, race, sex, place and date of birth, and place of initial hospitalization was analyzed. The most striking observation was the geographic distribution of the disease, revealing an endemic area in the eastern United States. To this degree, this study confirmed the earlier report on distribution made by Michael, *et al.* on a group of service men with sarcoidosis.

A review of ecologic factors which correlated with the distribution of this disease suggested that some aspect of the eastern forest distribution, where pine is the predominant tree, might be an important environmental factor related to the etiology of the disease. The distribution of birthplace of veteran patients diagnosed as having sarcoidosis correlated better with pine forests than it did with the sandy soil distribution suggested by Gentry, *et al.* as a possible factor related to the geographic concentration of patients with sarcoidoisis.

The epidemiologic studies led to a consideration of certain biologic and chemical materials associated with pine forests as possible etiologic agents. It was found that pine pollen is acid fast when exposed to the Ziehl-Neelsen stain, and that it contains certain chemical fractions similar to those of tubercle bacilli (diaminopimelic acid) and an acid-fast lipid. It also contains a phosphatide portion which evokes an epithelioid and giant cell response when injected intradermally or intraperitoneally into guinea pigs and rabbits. This response is similar to that observed by Sabin using tubercle bacillus phosphatide. Clinical evidence of a relationship between pine pollen and clinical sarcoidosis is still lacking. Indeed, there may prove to be many agents capable of inciting sarcoid granulomas.

T. H. NOEHREN

Epidemiologic and Clinical Aspects of Ornithosis (in German). H. MELZER.* *Deutsche med. Wchnschr.*, April 3, 1959, 84: 664–675. (*Städtische Krankenanstalten, Wuppertal-Elberfeld, W. Germany)

Twenty-eight proved and 9 probable cases of ornithosis are reported. In most instances the disease had been transmitted from pet parakeets, of which several were shown to harbor the virus. In the clinical features the variations in severity, the atypical appearance of the lung infiltrate, the relative bradycardia, and the frequent electrocardiographic abnormalities are stressed. Two elderly patients died of cardiovascular complications. Van den Bergh's test was often positive, the Wassermann test occasionally so. Although the complement fixation was always positive, the titer level varied greatly. Antibodies were usually demonstrable on the twelfth day of illness, but in one case as early as the eighth. In several instances, early administration of antimicrobials inhibited the expected rise in antibodies. In 2 cases there was a rapid fall in antibody titer by the third week of illness. It was often possible to demonstrate the virus in sputum during the first 2 weeks of illness. Broad-spectrum antimicrobials proved to be of value, although in 2 cases the fever relapsed even during treatment.

J. HAAPANEN

Talc Pneumoconiosis. A. Seeler,* J. Gryboski, and H. MacMahon. *A.M.A. Arch. Indust. H.*, April, 1959, 19: 392–402. (*Mt. Auburn Hosp., Cambridge, Mass.)

Two cases are described of pneumoconiosis occurring in rubber workers who were exposed to high concentrations of talc for about 25 years. The lungs in both cases showed extensive fibrosis which did not, however, show a specific pattern that might be of diagnostic value. In neither case were the classic laminated silicotic nodules encountered. In view of evidence such as these 2 cases and the other recent autopsy reports on pneumoconiosis occurring in talc workers, talc must be regarded as material which will cause pneumoconiosis if a high concentration of the dust is inhaled for many years.

T. H. Noehren

Observations on Non-attendance in a Community Chest X-ray Survey. W. B. Fletcher,* J. M. Mair, S. A. Sklaroff, and J. Williamson. *Tubercle*, April, 1959, 40: 82–92. (*Dept. of Health, Edinburgh, Scotland)

In a municipal ward of Edinburgh, with a resident population of 13,012 aged 15 years and older, an intensive chest roentgenogram campaign lasting 4 weeks produced a response of 83.4 per cent; a special survey carried out immediately thereafter, using full-time staff, raised this figure to 89.9 per cent. A significantly higher prevalence of pulmonary tuberculosis than in the main campaign was demonstrated in males of all ages filmed in the supplementary campaign; for active pulmonary tuberculosis this difference was significant only in males aged 35 to 64 years. In females, the only group in the supplementary survey which showed a significantly higher rate than that found in the main campaign was tuberculosis of doubtful activity among those aged 65 years and older.

Many people accustomed to being roentgenographed through their employment declined a film during the survey. Others who felt perfectly fit did not see any need for examination, and some cited press and magazine reports on radiation hazards. Another group of people insisted that they had been filmed in the main campaign, but were unable to produce the letter sent to each examinee. Frailty and unfitness, especially among the elderly, were readily identifiable causes of nonattendance, constituting 2 per cent of the total population at risk. By far the largest single group were those who gave no reason—4.3 per cent of the total population at risk. Specific objections to being roentgenographed were offered by only 0.9 per cent of the population at risk.

It is suggested that well-planned campaigns employing voluntary house visitors, coupled with special appeals—material and otherwise—to the older people are the most economic means at present of detecting pulmonary tuberculosis in an urban community. The speed at which tuberculosis morbidity declines in areas where such campaigns have been carried out will be the most appropriate measure of their success.

M. J. Small

Silicosis: A Critique of the Present Concept and a Proposal of Modifications. P. Gross.* *A.M.A. Arch. Indust. H.*, April, 1959, 19: 426–430. (*Mellon Institute, Pittsburgh, Penn.)

With the passage of time, many concepts become subject to modification. The present concept of pneumoconiosis, however, has remained virtually unchanged for more than 50 years, although in more recent times certain aspects of it have come under attack. It is the aim of this communication to depict the present concept of pneumoconiosis in general (and of silicosis in particular) diagrammatically as a flow chart, and to point out those aspects which have become incongruous in the light of modern knowledge.

The present, long-established concept of silicosis is reviewed and attention is focused upon certain incongruities. These include, among others, the necessity for postulating hitherto unknown and undescribed tropisms in order to account for the various elective migrational paths of macrophages. A new concept of silicosis is formulated which obviates the incongruities of the old concept. Both concepts are represented diagrammatically as flow charts.

T. H. Noehren

Silicosis in the Foundry—Medical Control. W. B. Ayre.* *A.M.A. Arch. Indust. H.*, May, 1959, 19: 490–496. (*1321 Canora Rd., Montreal, Quebec, Canada)

Silicosis in foundry workers may show variable disease patterns resulting from differences in exposure to silica dust according to occupation. In a chipper, the disease may be principally in the apices. Combined exposure to silica dust and iron oxide may result in siderosilicosis, complicating the roentgenographic picture and subsequent clinical assessment. Medical control of silicosis is concerned with those factors which may minimize the disability phase or delay its appearance. Most important is an effective program of tuberculosis control—casefinding and prompt treatment. It is suggested that with improved antimicrobial therapy, it might be rational to treat every case with conglomeration on a prophylactic basis. The importance of an

awareness of developing cardiac disease and the application of electrocardiography are stressed.

W. J. STEININGER

The Comparative Values of the Tuberculin and BCG Tests (in French). TH. BAUMANN. *Schweiz. Ztschr. Tuberk.*, No. 5, 1958, 15: 273–299.

On the basis of more than 100,000 persons tested, it is concluded that the Moro patch test, as well as the Mantoux test, in concentrations of 0.1 and 1.0 mg., are 100 per cent positive in cases of spontaneously infected or BCG-vaccinated individuals less than 50 years of age. For persons more than 50, the Mantoux tests are positive in 92 per cent of the cases.

The BCG test done in a concentration of 0.005 mg. (400,000 germs in Swiss vaccine) is equivalent to the 1.0 Mantoux. The BCG test can be employed only for testing BCG-vaccinated persons; it is not for general use since, in reality, it is a micro-vaccination apt to cause undesirable reactions in nonvaccinated individuals.

Z. VIRÁGH

The Chemical Composition of Weybridge PPD. D. S. P. PATTERSON.* *Tubercle*, April, 1959, 40: 71–81. (*Central Veterinary Lab., Weybridge, Surrey, England)

The active principle of Old Tuberculin which is precipitated by protein-precipitating reagents was originally described as a purified protein derivative (PPD) by Seibert and Munday. At the Central Veterinary Laboratory in Weybridge, Green adapted the procedure of Seibert to give a simple method for the large scale production of mammalian and avian-type PPD for use in the Ministry of Agriculture's scheme for the eradication of bovine tuberculosis in the United Kingdom. The protein is separated from steamed culture filtrates by precipitation with trichloracetic acid (TCA). Although many experimental PPD powders have been prepared either by freeze-drying or by dehydration with organic solvents, the tuberculoprotein routinely issued at Weybridge is at no stage of its preparation obtained in the dry state. Unlike some other products, however, Weybridge PPD is unusually stable to heat and storage and this, coupled with the fact that in the presence of tuberculous sera it is able to fix guinea pig complement, suggested the presence of additional nonprotein constituents. In the present paper, the results of the chemical analyses of several PPD powder preparations are given.

Chemical analyses of freeze-dried preparations of tuberculoprotein obtained from the heated culture filtrates of human and avian strains of mycobacteria showed that only about 63 per cent of the dry weight could be regarded as true protein. Small amounts of carbohydrate were present, but the majority of the nonprotein fraction was made up of nucleic acid and lipid soluble material.

The readily soluble lipid was composed of three main fractions. A methanol and water-soluble fraction, which represented about 9.5 per cent of the original weight of PPD powder, was not active as a complement-fixing antigen; neither did it elicit a significant response in the intradermal tuberculin test. Apart from a small protein contamination, this fraction appeared to consist mainly of intermediate molecular-weight organic acids. The remainder of the readily soluble lipid consisted of acetone-soluble fats and phosphatide, the latter being a highly active complement-fixing antigen. PPD powders prepared by drying TCA-precipitated tuberculoprotein with organic solvents had greater protein contents than the freeze-dried products (from 67 to 92 per cent), due to the partial extraction of lipids with the solvents during their preparation.

It is suggested that the analyses of the freeze-dried powders represent a fairly true chemical description of the actual PPD contained in the tuberculin solutions which are used in the diagnostic intradermal test. The nonprotein components of the PPD complex may be responsible for the high stability of Weybridge tuberculin.

M. J. SMALL

The Work of the British Royal Commission on Tuberculosis, 1901–1911. J. FRANCIS.* *Tubercle*, April, 1959, 40: 124–143. (*U. of Queensland, Brisbane, Australia)

At the turn of the century, tuberculosis was responsible for 20 per cent of all deaths and was the subject of great public interest. It was commonly believed that bovine tuberculosis was an important source of infection for man, and legislation was based on this belief. Robert Koch challenged this view in 1901 and a Royal Commission was appointed to "enquire into the relations of Human and Animal Tuberculosis." Its work was carried out between 1901 and 1911, during a period when successive international congresses on tuberculosis were the subject of great public interest and controversy: Koch maintained his original views with little alteration at the International Congress in 1908.

The Royal Commissioners and their assistants were unruffled by these events. The conclusions in their Reports, published from 1904 onwards and supported by massive appendices, are expressed in beautifully clear English and convey

an immediate impression of authority. None have had to be seriously modified. The Reports provide a mine of information on many aspects of tuberculosis in animals and man. They clearly established the existence of the three types of tubercle bacilli and their pathogenicity for a large variety of species. They thus showed to what extent "animals and man can be reciprocally infected with tuberculosis." These reports therefore provided a firm basis for all subsequent legislative and other action for the control of tuberculosis (Author's summary).

M. J. SMALL

Isoniazid in a Probable Role in Tuberculosis Control. E. FRIEDMAN.* *Arch. Pediat.*, June, 1959, 76: 237–242. (*416 Marlborough St., Boston, Mass.)

Most cases of active tuberculosis occur in the tuberculin-positive group. Preventorium care of these children consisting mainly of nonspecific measures, as shown in a study at the Prendergast Preventorium in Boston, proved to be beneficial in the prevention of adult type tuberculosis, but because of the large numbers involved and the expense that it would incur, it was found to be impractical. For this reason, a chemoprophylaxis program has been instituted at the Boston Tuberculosis Association. Those children who gave a definitely positive tuberculin reaction and had a history of massive exposure such as familial contagion, as contrasted with the so-called "street T. B." where the infection was transient and, of course, those who showed roentgenographic evidence of primary tuberculosis in the lung, were selected for treatment.

Up to the present, the numbers treated are too few and the length of time too short to warrant any conclusions. However, isoniazid was found to be highly effective in the prophylaxis of early complications of primary tuberculosis during the period of its administration. Those who showed roentgen evidence of infection showed a marked reduction in the film shadows, and isoniazid also seemed to have a definite effect, in some cases, on the reduction of the intensity of the tuberculin reaction.

M. J. SMALL

Deaths from Pulmonary Tuberculosis in Leicester during 1956 and 1957. J. P. ANDERSON.* *Tubercle*, April, 1959, 49: 99–105. (*Chest Unit, Isolation Hosp., Groby Road, Leicester, England)

The city of Leicester has shared in the national decline of mortality from pulmonary tuberculosis. The official figures of 28 deaths (0.095 per 1,000) in 1956, and the same number in 1957 (0.096 per 1,000) are the lowest ever recorded in the city. The present study is an attempt to discover how the position may be improved still further, for every death from pulmonary tuberculosis must now be considered a failure of preventive and therapeutic medicine. This study shows that a further reduction in the recorded mortality from pulmonary tuberculosis is possible. Greater accuracy in death certification should be a first step. A general guiding principle should be that the mere presence of pulmonary tuberculosis is no reason nowadays for automatically quoting it as a primary cause of death.

Older men with pulmonary tuberculosis fared worse than other age and sex groups in this series: they were diagnosed later; they died sooner and in larger numbers; they were not easy to treat; they frequently had chronic bronchitis, emphysema and, later, cor pulmonale, to say nothing of bronchial carcinoma. Earlier diagnosis is imperative in this group for any improvement in prognosis. More routine presymptomatic roentgenography, especially in hospital and industry, is urgently needed. Some patients in this series suffered overlong delay before treatment was started. Often it is justifiable to treat on suspicion and "diagnose" the activity in retrospect. All too often, "late" treatment is also required: several of the patients with very extensive disease were kept alive more than 2 years by continuous chemotherapy. In these (and other) patients, the occasional emergence of drug-resistant tubercle bacilli is a grave clinical and public health problem. But no instance of primary infection with such organisms has yet been encountered in Leicester.

Surgical treatment can still play a part in reducing pulmonary tuberculosis mortality. But those patients most needing such help often have an inadequate cardiorespiratory reserve. The great importance of the "soil" infected by the tuberculous "seed" was clearly seen in older men. Sustained treatment of bronchitis and heart failure could lower "tuberculous" mortality in this group. And curtailment of smoking is nowhere more vital than here, for the indirect evidence in this report supports the findings of others that cigarette smoking is a significant factor in initiating breakdown of old tuberculous lesions in men.

M. J. SMALL

Microdetermination of Toluenediisocyanates in Atmosphere. K. MARCALI.* *A.M.A. Arch. Indust. H.*, March, 1959, 19: 354. (*E. I. duPont de Nemours Co., Wilmington, Del.)

The extensive application of toluenediisocyanates has become of increasing importance in the last few years. Preliminary toxicologic studies

have indicated that the maximal allowable concentration of toluenediisocyanate in air may be 0.1 ppm. Exposure to higher concentrations may cause serious respiratory disorders. A sensitive and rapid analytic method has been developed for the determination of toxic quantities of toluenediisocyanates in air. This diazometric method has been incorporated into a compact, portable analytic kit.

T. H. NOEHREN

ROENTGENOGRAPHY

Left Atrial Calcification. C. W. VICKERS,* O. W. KINCAID, F. H. ELLIS, JR., and A. J. BRUWER. *Radiology*, April, 1959, 72: 569–575. (*Mayo Clinic, Rochester, Minn.)

Calcification of the left atrial wall has been diagnosed roentgenographically in 5 cases at the Mayo Clinic since 1953. The finding is pathognomonic of severe rheumatic heart disease of long standing. It may or may not be associated with mitral insufficiency. The presence of large amounts of calcium in the wall of the left atrium presents technical problems for the surgeon which may increase the hazard of operation. Its recognition is of importance, therefore, in establishing the decision of operability.

W. J. STEININGER

Great Vessel Involvement in Lung Cancer: Angiocardiographic Report on 250 Consecutive Proved Cases. I. STEINBERG* and N. FINBY. *Am. J. Roentgenol.*, May, 1959, 81: 807–818. (*N. Y. Hosp.-Cornell Med. Center, N.Y., N.Y.)

Angiocardiographic study of 250 consecutive cases of primary cancer of the lung disclosed that 30 per cent involved the great vessels (superior vena caval and central pulmonary arterial) and were therefore nonresectable. These cases were chiefly due to hilar and mediastinal involvement. In over 50 per cent of the cases, lobar and segmental pulmonary arterial involvement were demonstrated, thus permitting preoperative evaluation of the cardiovascular system. In some instances, because the vascular deformity was confined to a segment or lobe, lobectomy rather than pneumonectomy seemed feasible. Finally, with bilateral pulmonary disease, angiocardiography provided a precise evaluation of the pulmonary circulation. Thus, pneumonectomy in a potentially respiratory-crippled patient was avoided.

For the proper evaluation of the cardiovascular system in patients with lung cancer, scrupulous attention to the roentgenographic technique of angiocardiography is essential. Frontal (erect) angiocardiograms for visualization of the superior vena caval and pulmonary arterial circulation are mandatory. If there is involvement of the main branches of the pulmonary artery, oblique studies are also indicated. The right anterior oblique position should be used when there is deformity of the right pulmonary artery; the left view when the left pulmonary artery is involved. Then only will angiocardiography achieve its maximal usefulness in determining resectability and prognosis (Authors' summary).

T. H. NOEHREN

Barium Sulfate and Bismuth Subcarbonate Suspensions as Bronchographic Contrast Media. S. W. NELSON,* A. CHRISTOFORIDIS, and P. C. PRATT. *Radiology*, June, 1959, 72: 829–838. (*Ohio State U. Health Center, Columbus)

Experimental and clinical studies show that barium sulfate (Micropaque) suspended in normal saline which contains at least 1.5 per cent sodium carboxymethylcellulose is a safe bronchographic contrast material. There is no evidence of sensitivity. Barium-sulfate bronchography in 50 patients was attended by fewer instances of temperature and pulse elevation than observed in a parallel group of 50 patients in whom propyliodine (Dionosil Oily®) was used. The low surface tension characteristic of this aqueous, yet viscous, solution resulted in excellent and uniform coating of the bronchial mucosa. The roentgenographic contrast and density were at least equal to those obtained with iodized bronchographic contrast materials. Elimination of barium sulfate was rapid except in 3 (of 50) patients with severe emphysema. There was no evidence of chronic fibrosis or foreign-body granulomas resulting from use of barium or sodium carboxymethylcellulose as shown by animal experiments and by histopathologic study of resected pulmonary tissue from 15 patients after barium-sulfate bronchography. Bismuth subcarbonate suspensions are not considered safe bronchographic media, since histopathologic studies of animal lungs showed severe changes as long as 10 weeks following injection into the tracheobronchial tree.

W. J. STEININGER

Cricothyroid Bronchography With a Polyethylene Catheter. J. WILLSON.* *Am. J. Roentgenol.*, February, 1959, 81: 305–311. (*Johns Hopkins Hosp., Baltimore, Md.)

There is a need for an alternative method of bronchography when tracheal intubation is difficult or impossible, and the several advantages of the cricothyroid procedure by direct needle puncture seem worth preserving. These considerations led to the development of a modification of the technique which eliminates most of the dis-

advantages of the method while preserving its advantages. A small polyethylene catheter is inserted into the trachea through the needle, then the needle is immediately removed. Bronchograms can thus be taken in any position desired. There is no discomfort to the patient from the tube.

Bronchography has been performed by this method in 25 patients in the departments of radiology of The Johns Hopkins Hospital and Union Memorial Hospital. The results were excellent and there were no complications. It has proved to be a valuable adjunct to the other methods of bronchography.

T. H. NOEHREN

Roentgen Findings in Aneurysm of the Descending Thoracic Aorta. J. G. DAVIS* and P. WINSOR. *Am. J. Roentgenol.*, May, 1959, 81: 819–825. (*Wadsworth General Hosp., Los Angeles, Calif.)

Seven cases of aneurysm of the descending thoracic aorta are reported; 3 cases were syphilitic and 4 were arteriosclerotic. Four cases were subjected to thoracotomy. Of these, 2 were correctly diagnosed preoperatively, the third was thought to have a bronchogenic cyst, and the fourth a bronchogenic carcinoma. In the 3 remaining cases, the correct diagnosis was made on routine roentgenographic examinations.

Five of the 7 cases exhibited on the postero-anterior roentgenogram of the chest a mass in the hilar area, 1 on the right side and the other 4 on the left side. In another case, the aneurysm simulated an enlarged pulmonary artery segment. The last case showed a mass in the right cardiophrenic angle. Roentgen studies, including retrograde aortography, angiocardiography, and laminagraphy will, in most instances, establish the diagnosis of aneurysm of the descending thoracic aorta (after authors' summary).

T. H. NOEHREN

Pleural Effusions Following Supervoltage Radiation for Breast Carcinoma. A. L. BACHMAN* and K. MACKEN. *Radiology*, May, 1959, 72: 699–709. (*Francis Delafield Hosp., N.Y., N.Y.)

Pleural effusions most probably due to radiation were observed in 5 per cent of about 200 cases of carcinoma of the breast treated with 2-million-volt radiotherapy. These observations were helpful in differentiating radiation and metastatic effusions: (*1*) In all instances radiation effusions appeared within 6 months, often within 2 to 4 months, following completion of radiotherapy. (*2*) The effusion was always accompanied by a radiation pneumonitis. (*3*) Spontaneous disappearance of fluid indicated it was probably due to radiation. (*4*) Disappearance or persistence of fluid followed by sudden reappearance or increase was due to metastasis. Roentgenographic appearance and persistence of effusions were not helpful in differentiating the two conditions.

W. J. STEININGER

Subpleural Interlobular Lymphectasia Reflecting Metastatic Carcinoma. B. LEVIN.* *Radiology*, May, 1959, 72: 682–688. (*Michael Reese Hosp., Chicago, Ill.)

One of the causes of subpleural pulmonary lymphatic dilatation is obstruction and distention of these vessels by tumor cells. The dilated lymphatics may become visible on chest roentgenograms in the form of short transverse lines in the bases of the lungs, permitting the diagnosis of metastatic carcinoma. There are relatively few other conditions which cause distention of the subpleural lymphatics, and these usually can be readily excluded. Five illustrative case histories and roentgenograms are presented.

W. J. STEININGER

The Radiographic Evaluation of Pulmonary Vasculature in Children with Congenital Cardiovascular Disease. D. C. ARNOIS,* F. N. SILVERMAN, and M. E. TURNER. *Radiology*, May, 1959, 72: 689–698. (*U. of Cincinnati College of Med., Ohio)

In a study of the ability of roentgenologists to recognize increased or decreased pulmonary vascularity in conventional roentgenograms of the chest in children with congenital cardiovascular malformations of a known nature, there was a distinct difference in the number of errors related to the recognition of pulmonary undervascularity in comparison with the recognition of pulmonary overvascularity. Apparently it is much easier to recognize pulmonary overvascularity than undervascularity in children with congenital malformations of the heart.

W. J. STEININGER

Pulmonary Manifestations in Collagen Diseases. C. NICE, JR.,* A. MENON, and L. RIGLER. *Am. J. Roentgenol*, February, 1959, 81: 264–278. (*U. of Minnesota Hospitals, Minneapolis)

The roentgen findings produced in the chest by five collagen diseases are presented. Selected case reports are given to illustrate how an analysis of various combinations of nonspecific roentgen signs may lead one to suspect a collagen disease, and occasionally make a specific diagnosis (Authors' summary).

T. H. NOEHREN

The Importance of Chest Roentgenography in the Diagnosis of Pulmonary Embolism. G. Stein,* J. Chen, F. Goldstein, H. Israel, and A. Finkelstein. *Am. J. Roentgenol.*, February, 1959, 81: 255–263. (*Graduate Hosp. of U. of Pennsylvania, Philadelphia)

The roentgenographic changes observed in 72 patients following pulmonary embolism are presented. Abnormalities were noted in 60 instances. Pleural effusions (33 instances) and homogeneous densities resembling those produced by pneumonia (27 instances) were the most common findings. Elevation of the diaphragm was noted in 21 patients, usually in association with some parenchymal abnormality. Linear densities, presumably representing focal areas of atelectasis or small interlobar effusions, were observed in 19 patients.

Densities appeared rapidly following embolism. Abnormal findings were observed as frequently in roentgenograms made within 24 hours of the onset of symptoms as in those made subsequently. Densities resulting from embolism were slow to resolve. Abnormal findings cleared within a week in 5 cases, and persisted for more than 4 weeks in 19 cases.

The correct diagnosis was suggested by the roentgenologist in 10 cases in which the possibility of pulmonary embolism had not been clinically considered. The diagnosis of embolism was suggested by the roentgenologist in only 54 per cent of the entire series. The failure to consider this diagnosis in the other cases must be attributed to misconceptions concerning the roentgenographic criteria for diagnosis of pulmonary embolism and lack of familiarity with the clinical manifestations of this disease.

T. H. Noehren

An Early, Hitherto Unrecognized, Simple Roentgenographic Aid in the Diagnosis of Major Pulmonary Embolism. L. A. Soloff* and J. Zatuchni. *Am. J. M. Sc.*, May, 1959, 237: 608–611. (*Temple U. School of Med., Philadelphia, Penn.)

The mid-right (Mr) diameter of the cardiac silhouette, rarely exceeding 50 mm. in the normal, is the greatest horizontal distance between the midspinal line and the right border of the cardiac silhouette in the frontal plane. A case is presented in which the Mr diameter, normal upon admission to the hospital, increased greatly after pulmonary embolism occurred. The right border of the cardiac silhouette became more convex and bulged to the right. It is suggested that an increase in the Mr diameter with bulging of the right border of the cardiac silhouette may be the sole initial roentgenographic finding of major pulmonary embolism.

W. J. Steininger

Arteriovenous Fistula of the Lung. B. P. Sammons.* *Radiology*, May, 1959, 72: 710–715. (*U.S. Naval Hosp., St. Albans, N.Y.)

The increasingly frequent detection of pulmonary lesions necessitates the routine inclusion of pulmonary arteriovenous fistula in the differential diagnosis. The presence of "feeders" in the region of the lesion strongly suggests its possibility. Fluoroscopy, tomography, and angiocardiography will detect all but minute pulmonary telangiectasias and will give the necessary information for proper surgical attack. Symptoms consist of dyspnea, cyanosis, and clubbing of digits. Examination may reveal polycythemia, a murmur over the pulmonary lesion, and telangiectasia of skin and mucous membranes. Complications of hemoptysis, meningoencephalitis, cerebral thrombosis, and brain abscess may be presenting complaints. Because of the occurrence of these extremely serious complications, surgery is the treatment of choice even in asymptomatic individuals (Author's summary).

W. J. Steininger

Roentgen Aspects of Blastomycosis. W. Boswell.* *Am. J. Roentgenol.*, February, 1959, 81: 224–230. (*Lexington, Ky.)

North American blastomycosis, more commonly called blastomycosis, is a chronic infectious disease caused by the organism *Blastomyces dermatitidis*. The disease is characterized by the formation of suppurative, as well as granulomatous, lesions in any part of the body. The skin, lungs, and skeletal system are involved most frequently.

Blastomycosis may exist in two forms, cutaneous and systemic. The cutaneous type presents as a chronic or subacute ulcerating process. The systemic type is characterized by pulmonary infection with wide-spread distribution of lesions in the subcutaneous tissues and skeletal system, less frequently in the internal viscera, and at times in the central nervous system. Patients with pulmonary involvement usually develop cutaneous lesions. Many patients with so-called cutaneous forms of the disease have abnormal roentgen findings in the lungs.

The systemic form usually begins insidiously with symptoms suggestive of respiratory infection. The patient may have a low-grade fever, cough, and chest pain. As the pulmonary lesion progresses, the patient may develop malaise, sweats, and hemoptysis. Dissemination of the fungus

usually occurs by the hematogenous route. The patients with blastomycosis of bone may present themselves because of a chronic draining sinus of the subcutaneous or deep tissues.

The roentgenographic findings in pulmonary blastomycosis are not uniform or characteristic. In fact, the infection may manifest itself in the lungs as disseminated miliary granulomas, homogenous consolidation, solitary or multiple nodular densities, abscesses, or fibrotic lesions. Hilar or mediastinal lymph node enlargement, as well as pleural involvement, may be seen. The roentgen findings of 5 patients with systemic blastomycosis with follow-up roentgenograms form the basis for this report.

T. H. Noehren

Pulmonary Paragonimiasis. An Evaluation of Roentgen Findings in 38 Positive Sputum Patients in an Endemic Area in Thailand. R. Suwanik* and C. Harinsuta. *Am. J. Roentgenol.*, February, 1959, 81: 236–244. (*Siriraj Med. School, Dhonburi, Thailand)

Pulmonary paragonimiasis is a disease caused by the presence within the lung parenchyma of the oriental lung fluke, *Paragonimus westermani*. In 1957, a survey for Paragonimus infestation was undertaken in a district of Saraburi Province, Thailand. A series of 140 persons with chronic cough as the chief complaint were examined. Positive sputum for ova of *Paragonimus westermani* was found in 38 cases. Chest roentgenograms were taken of these 38 patients and the lung findings studied. This article presents an evaluation of the chest roentgen findings in these 38 cases.

Patients whose sputum was positive for *P. westermani* ova presented three cardinal symptoms: repeated blood-stained sputum, chronic cough, and chest pain. One patient exhibited creeping subcutaneous eruptions on his face; another had cerebral symptoms. Only one had ova of the parasite demonstrated in the feces. The age distribution ranged from 11 to 54 years, the average being 30 years. The ratio between males and females was approximately 5 to 3. Symptoms were present over a period of from 6 months to 5 years. The average count of the eosinophils in the peripheral blood was 15 per cent. No pleural fluid was noted in any of the cases. Only 2 patients had intradermal tests for paragonimiasis, using the antigen prepared from the adult worm; both were positive.

Abnormal roentgen findings in the chest were encountered in 36 of the 38 cases studied. These were classified into four types: (*1*) characteristic ring shadows; (*2*) opacities with poorly defined borders; (*3*) linear infiltration; and (*4*) pleural thickening. The changes varied according to the stage of the disease, and often there was an intermingling of several types on the same roentgenogram.

Since roentgen abnormalities were encountered in 95 per cent of patients having positive *P. westermani* sputa, it is evident that a roentgenographic examination of the chest is one of the best means of establishing the definitive diagnosis, estimating the extent of the disease, and observing its subsequent course. A chest film should also be utilized as a screening procedure.

T. H. Noehren

Unusual Roentgen Manifestations of Primary Staphylococcal Pneumonia in Infants and Young Children. G. Schultze.* *Am. J. Roentgenol.*, February, 1959, 81: 290–295. (*Johns Hopkins Hosp., Baltimore, Md.)

This paper reports 41 cases of staphylococcal pneumonia seen at the Johns Hopkins Hospital during the period between 1955 and 1957, inclusive. Primary staphylococcal pneumonia is defined as staphylococcal pneumonia arising in the absence of pre-existing pulmonary disease; it has become a common and important entity. Fortunately, staphylococcal pneumonia, especially in children less than one year of age, is one of the few lung diseases in which early roentgen manifestations appear to have pathognomonic significance. The reliability of these signs has been emphasized by several investigators.

Typical roentgen features: (*1*) Localized parenchymal infiltrations: These are often of lobar distribution and involve 1 to 3 lobes of the same side. Bilateral involvement occurred only 5 times in this series of 41 cases, and in 4 of these it was minimal and transient. Several recent cases suggest that, as in adults, it may be more common in older children. Mediastinal lymph node enlargement is unusual. (*2*) Pleural involvement: Most frequently, this takes the form of an empyema. In this series empyema was present in approximately 90 per cent of the cases. Also observed were spontaneous pneumothorax and pyopneumothorax, often of the tension types, in approximately 25 per cent of the patients. (*3*) Development of pneumatoceles: Pneumatoceles are emphysematous cysts or cavities probably arising from small areas of necrosis located in the peribronchial interstitial tissue. These areas communicate with bronchi through small sinuses and are inflated rapidly by check-valve action of the edematous bronchial mucosa or luminal secretions. They usually form in the most densely infiltrated areas, are thin-walled, and sometimes

contain fluid. During the acute stage of the disease they often, within hours, change considerably in number, size, and position. While they may be as small as 0.5 cm. in diameter, they may expand or coalesce to form very large cysts indistinguishable from and producing effects similar to tension pneumothorax. This is especially true if they are situated anteriorly.

Marked parenchymal and pleural change may remain after complete clinical recovery from the disease; usually, however, this disappears within 2 weeks to 2 months, and corrective surgery should not be undertaken prematurely. The average period from onset to complete healing of the disease has been approximately 4 weeks. Not infrequently, residual, asymptomatic pneumatoceles persist for longer periods; a few, for periods of 6 to 8 months.

In a number of cases (approximately 15 per cent), the early picture was atypical and the roentgen diagnosis of staphylococcal pneumonia was not made at the onset. The unusual features were: unilateral and bilateral spheroid lesions; early nonspecific changes; hilar lymph adenopathy, and massive atelectasis. These cases are described in some detail.

T. H. NOEHREN

Roentgen Diagnosis of Anomalous Pulmonary Venous Drainage of Right Lung Into Inferior Vena Cava. I. STEINBERG.* *Am. J. Roentgenol.*, February, 1959, 81: 280–289. (*New York Hosp.–Cornell Med. Center, N. Y., N.Y.)

The purpose of this paper is to report 3 new cases of anomalous pulmonary venous drainage of the right lung into the inferior vena cava, each with an unusual feature, to review the experience of 9 cases previously studied, and to re-emphasize the importance of roentgenography in diagnosis.

Roentgenography: A common feature, observed in all but one case of anomalous pulmonary venous drainage of the right lung into the inferior vena cava, is the appearance in the conventional frontal roentgenogram of a broad, multiple-branching channel curving in crescentric fashion near the right cardiac border and descending toward the right cardiohepatic region. Often, the right lung is hypoplastic with narrowed intercostal spaces, while the left lung is voluminous and plethoric. In 5 instances the right atrium was prominent and projected well into the right hemithorax. The cases with pronounced rotation of the heart into the right hemithorax also had the characteristic vascular deformity adjacent to the right cardiac silhouette.

Angiocardiography: By visualizing the course of the right anomalous pulmonary venous system as it enters the inferior vena cava, this method provides the definitive diagnosis of right pulmonary venous drainage into the inferior vena cava. Asymptomatic patients with partial anomalous drainage of the right lung into the inferior vena cava do not require treatment, this type of anomaly being compatible with long life. However, patients with dyspnea, recurrent pulmonary infections, and plethora of the lungs may have an associated cardiac lesion, such as an atrial septal defect that requires repair.

T. H. NOEHREN

Positive Roentgenograms Plus Positive Sputum Smears Do Not Always Equal Pulmonary Tuberculosis. C. GOOD,* D. CARR, and L. WEED. *Am. J. Roentgenol.*, February, 1959, 81: 187–195. (*Mayo Foundation, Rochester, Minn.)

In order to emphasize again that the roentgenographic diagnosis of pulmonary tuberculosis is presumptive and should be confirmed by identification of the specific microorganism by means of its cultural characteristics and virulence for laboratory animals, a report of 7 cases is presented in which positive film and sputum smear results existed. The presence of acid-fast bacilli in a stained sputum smear does not necessarily confirm a roentgenographic impression of pulmonary tuberculosis.

T. H. NOEHREN

The Changing Roentgenologic Picture in Pulmonary Tuberculosis Under Modern Chemotherapy. R. FRIEDENBERG,* N. ISAACS, and M. ELKIN. *Am. J. Roentgenol.*, February, 1959, 81: 196–205. (*Albert Einstein College of Med., N. Y., N. Y.)

Prior to chemotherapy, the mortality rate of patients with tuberculous cavities or acute miliary disease was greater than 50 per cent in 2 years, and approximated 100 per cent in 5 to 10 years. Cavity closure in large series varied from 5 per cent to 10 per cent. Nearly 50 per cent of arrested cases were destined to suffer relapse. With present techniques, survival rates approximate 90 per cent in acute disease.

Cases have been selected to illustrate the rapid, marked roentgenographic improvement which may occur in a short period of time after chemotherapy. Productive, exudative, miliary, pneumonic, and cavitary forms of tuberculosis respond dramatically, if marked fibrosis and caseation have not occurred. Many long-standing cases of chronic disease without extensive fibrosis show rapid and marked improvement under proper therapy. The extent of involvement does not

affect the prognosis of the disease today as it did before modern antimicrobial therapy. The presence, however, of abnormal anatomic changes of extensive fibrosis or caseation must still be regarded as poor prognostic signs.

T. H. NOEHREN

Cervicothoracic Tumors: A Technical Aid to Their Roentgenologic Localization. F. RUNDLE,* R. DELAMBERT, and R. EPPS. *Am. J. Roentgenol.*, February, 1959, 81: 316–321. (*Royal North Shore Hosp., Sydney, Australia)

Mediastinal masses protrude into the lung fields, which are of a quite different order of density and against which tumor masses are usually clearly outlined. However, because of the obliquity of the thoracic inlet, about one-third of the superior mediastinum, the posterior and upper segment, is overlaid by cervical structures in conventional anteroposterior roentgenograms. Therefore, soft tissue masses in this area tend to be obscured and overlooked. If visualized, it is impossible without other evidence to determine whether they are cervical or thoracic in location. The upper and posterior portion of the superior mediastinum is also the most difficult in which to demonstrate soft tissue tumors clearly in lateral roentgenograms.

By tilting the patient into a position of exaggerated lordosis, excellent delineation of mediastinal masses in this obscure area can be obtained against a background of lung. The film is "shot" in a plane below the clavicles and vascular arches of the head, neck, and superior extremities, and through the open louvers of the upper ribs which are seen edge-on (Authors' summary).

T. H. NOEHREN

Less Familiar Roentgen Patterns of Pulmonary Granulomas. Sarcoidosis, Histoplasmosis and Noninfectious Necrotizing Granulomatosis (Wegener's Syndrome). B. FELSON.* *Am. J. Roentgenol.*, February, 1959, 81: 211–223. (*U. of Cincinnati, Ohio)

This report deals with three types of pulmonary granulomas: sarcoidosis, histoplasmosis, and noninfectious necrotizing granulomatosis. An attempt has been made to select material and to emphasize aspects of these diseases which are not familiar to many roentgenologists.

Under the heading of sarcoidosis is a description of two interesting roentgen patterns, one consisting of multiple large nodules, the other of multiple cysts. The subject of histoplasmosis is discussed with particular reference to the fibrocavitary form and to two unusual manifestations of the histoplasmoma growth and multiplicity. Evidence is presented to indicate that, in the endemic area, broncholithiasis, esophageal traction diverticulum, "middle lobe syndrome," and chronic superior vena caval obstruction may represent late sequelae of chronic histoplasmic tracheobronchial lymphadenitis (Authors' summary).

T. H. NOEHREN

Television Contrast Expansions of Single Roentgenograms. J. COHEN* and J. FISHER. *Am. J. Roentgenol.*, February, 1959, 81: 325–327. (*Albert Einstein Med. Center, Philadelphia, Penn.)

The principles of a television technique have been described for multiple contrast enhancements of various levels of luminance facilitating interpretation of single roentgenograms. Re-examinations or multiple exposures for different contrasts are obviated since enhancement in contrast is obtained with these television techniques over all regions of a single film, whether over- or underexposed (Authors' summary).

T. H. NOEHREN

The Radiologist Looks at Radiation Hazards. P. C. HODGES.* *Radiology*, April, 1959, 72: 481–488. (*U. of Chicago, Ill.)

It has not been shown, and in the opinion of the author it never will be shown, that the extremely small tissue doses delivered in properly conducted roentgen examinations increase the mutation rate or the incidence of leukemia or shorten the life span, but there is every reason to believe that with larger doses in man, as in laboratory animals, these effects will be produced. Therefore, every practical precaution should be taken toward keeping such doses at a minimum, doing what is possible to allay hysteria, and seeking always to establish the point that when there is genuine clinical need for roentgen examination and when that examination is done by a competent roentgenologist, the health risk to the patient and his progeny can be kept very small relative to the risk of getting along without the examination. Voltage, filtration, film and screen speed, minimal exposure, and full development are important factors in keeping tissue dose at a minimum. The use of rectangular ports greatly reduces useless radiation and is of particular importance in fluoroscopy and spot-filming.

W. J. STEININGER

The Government Looks at Radiation Hazards. C. C. POWELL.* *Radiology*, April, 1959, 72: 489–492. (*National Institutes of Health, Bethesda, Md.)

Only through mutual cooperation between the individual practitioner, his organizations, and official government agencies, is a reasonable and

appropriate approach to the problem of radiation hazards likely. Wholehearted cooperation and active participation of roentgenologists in the programs of governmental agencies are among the best ways to assure the avoidance of ill-advised and ill-considered action. The government in return can be of help in the support of research programs, in public education, in the maintenance of equipment in the safest operation condition, and in providing a legal basis for exclusion of the untrained and incompetent individual from using potentially hazardous equipment.

W. J. STEININGER

A Geneticist Looks at the Radiation Hazard. W. J. SCHULL.* *Radiology*, April, 1959, 72: 522–534. (*U. of Michigan Med. School, Ann Arbor)

Plant and animal experimentation point to certain radiogenetic principles. Chief among them are that the yield in gene mutations is linearly related to dosage, and it is independent of the intensity of the exposure, i.e., the effects of repeated exposure are cumulative. Recent demonstration of the apparent dependence in the mouse (and hence possibly in man) of yield of mutations on intensity of exposure does not necessarily alter the basic problem, although, if true, it would necessitate re-evaluation of our notions as to the magnitude of the risk. The average 30-year dose of ionizing radiation in the United States approximates 7 to 8 rads, with medical use accounting for over half of this amount. Six published appraisals of genetic hazards show no significant differences between exposed and nonexposed populations with respect to mortality, birth weight, anthropometry, and congenital malformation following parental exposure. An effect on sex ratio consistent with genetic notions has been observed, but there is reluctance to assert that the results *must* be due to the operation of sex-linked lethal mutants. More work and fewer speculations on genetic hazards are needed. Wisdom now dictates a conservative approach, limiting ionizing exposure as much as is consistent with the practice of good medicine.

W. J. STEININGER

Radiation Reduction by Combined Copper and Aluminum Filtration in Radiography. L. E. ETTER,* A. G. KAMMER, J. A. DATTOLI, H. CEMBER, C. R. GRIFFITH, and L. C. CROSS. *Radiology*, June, 1959, 72: 863–867. (*U. of Pittsburgh, Penn.)

From the roentgenographic standpoint, in many areas of examination a better range of tonal quality can be achieved by a combination of copper and aluminum filtration in the X-ray beam. With this improvement there is a tremendous reduction in radiation dosage to the skin and, incidentally, a resultant lowered depth dosage made possible by the higher quality beam. This results in lower bone-marrow dose as well as decreased dosage to the gonads, and is especially significant in conventional fluoroscopy, image amplification fluoroscopy, and cinefluoroscopy.

W. J. STEININGER

CHEMOTHERAPY

Remission in Disseminated Coccidioidomycosis Produced by Amphotericin B. J. COLWELL.* *Ann. Int. Med.*, April, 1959, 50: 1028–1035. (*U. S. Air Force Hosp., Tucson, Ariz.)

Therapy has not previously altered the relentless progression of disseminated coccidioidomycosis. More than a 50 per cent mortality rate continues despite treatment with a variety of agents. In the last year, a new antimicrobial, amphotericin B, has been released to clinical investigators. *In vitro* studies have shown good antifungal properties of this (Streptomyces) derivative. Encouraging results have been described in studies with animals. Several reports of effectiveness in disseminated coccidioidomycosis have recently appeared. The present paper describes a mycologic and clinical remission in a case of disseminated coccidioidomycosis produced by prolonged intravenous therapy with amphotericin B. Evidence of effectiveness was a temporary improvement after one month of treatment, followed by a relapse which was responsive to 28 weeks of intravenous therapy.

Toxic effects of the drug were confined to temporary elevation of the blood urea nitrogen and occasional casts in the urine. No permanent renal damage developed. There was no evidence of a toxic effect on the liver or bone marrow. The experience in the present case and a review of the literature to date suggest that adequate doses of amphotericin B (at least 1 mg. per kg. intravenously) must be administered for a prolonged period of time to achieve a remission in disseminated coccidioidomycosis.

T. H. NOEHREN

Prophylactic Chemotherapy in Chronic Bronchitis. E. N. MOYES* and Z. KALINOWSKI. *Tubercle*, April, 1959, 40: 112–118. (*Royal Infirmary, Worcester, England)

Ninety-eight cases of moderate or severe chronic bronchitis were included in a trial designed to compare 3 other chemotherapeutic regimes with continuous daily tetracycline during 4 months of the 1957–1958 winter. The results obtained in 93 patients divided into 4 treatment groups are described.

Clinical improvement was noted in 15 of 25 patients who received 750 mg. of tetracycline daily during the trial. Similar benefit was obtained in 10 of 21 patients who were given short courses of tetracycline, 1 gm. daily for 5 days, taken on their own initiative at the onset of exacerbations. With both methods there was a considerable reduction in the time lost from work compared with the previous winter. Although continuous daily tetracycline therapy may be preferable in certain advanced cases, intermittent treatment, as here described, offers a reasonable and less costly alternative with a wider field of application among sufferers from this disease.

Forty-seven patients, divided into 2 groups, were given short courses of erythromycin either for the prompt treatment of exacerbations, or at monthly intervals. It would appear from the results that this antimicrobial has no place in the routine management of chronic bronchitis.

A tetracycline-resistant strain of *Staphylococcus pyogenes*—susceptible to erythromycin—was isolated in 2 cases. In one it emerged after 3 months of daily tetracycline administration; in the other it was found in the pretreatment sputum and persisted in subsequent specimens. No resistant strains of *Haemophilus influenzae* or *Streptococcus pneumoniae* appeared.

This treatment is only justified in a relatively small proportion of the bronchitic population, those liable to frequent exacerbations, and with sputum that is predominantly or frequently purulent. The results suggest that the average bronchitic patient will require about two courses each winter. If the symptoms of an attack persist in spite of treatment and the sputum remains purulent, it should be cultured to discern the nature and antimicrobial susceptibility of the bacterial content before further treatment is prescribed. Except in these circumstances, there is no indication for routine bacteriologic examination of the sputum, for the results are rarely of value in predicting success or failure.

M. J. SMALL

Allergic Asthma Caused by Isoniazid (in German). U. BAUMGARTNER.* *Schweiz. med. Wchnschr.*, April 25, 1959, 89: 459–460. (*Davos, Switzerland)

Allergic rhinitis and bronchial asthma appeared in a 21-year-old tuberculous female during treatment with isoniazid. The scratch test with isoniazid 1:50 was positive. The asthma ceased following discontinuation of the drug.

J. HAAPANEN

Calcium Benzoyl PAS. Leading Article.* *Tubercle*, April, 1959, 40: 133–135. (**Tubercle*, Mandeville Place, W.1, London)

The main evidence on which the use of calcium B-PAS is being advocated is summarized. It may be as effective as sodium PAS in delaying the emergence of streptomycin- and isoniazid-resistant bacilli, but the evidence for this is not clear cut. It is said that calcium B-PAS is more pleasant to take than the sodium salt of PAS, and less likely to cause nausea, vomiting, and diarrhea. It may be accepted that it is less unpleasant to take, but even the evidence on the lower incidence of side effects is conflicting. Calcium B-PAS costs approximately twice as much as sodium PAS in Great Britain. It is on evidence such as this that calcium B-PAS is now being widely used.

M. J. SMALL

Chemotherapy of Tuberculosis. Editorial. *New England J. Med.*, June 25, 1959, 260: 1345.

In these days when there is so much concern about the difficulties that beset the medical profession and the public, as well as the manufacturers, in obtaining reliable evaluations of new chemotherapeutic agents, the Veterans Administration is saluted for its valiant and persistent efforts to obtain dependable answers to the practical questions arising in the use of chemotherapeutic and other measures in the management of tuberculosis. Physicians interested in the treatment of patients with tuberculosis have come to look forward each year to the meticulously controlled studies being carried out in the large number of hospitals of the V. A. and those of the Armed Forces that cooperate in these evaluations to bring them up to date in this field. The most recent conference, the eighteenth, at which the data from the cooperating hospitals were presented and discussed, took place early in February, 1959. A summary of the results of these deliberations follows.

M. J. SMALL

The Eighteenth Veterans Administration–Armed Forces Conference on the Chemotherapy of Tuberculosis. E. ROTHSTEIN.* *New England J. Med.*, June 25, 1959, 260: 1319–1321. (*V. A. Hosp., Brockton, Mass.)

General conclusions about so diversified a group of papers, and so protean a disease, are of course hazardous. It is apparent that the antituberculous effect of isoniazid is so great that its use is indicated in every case in which susceptible tubercle bacilli are found. In the absence of gross caseation and destruction, the inclusion of other drugs

adds little to the results achievable. In the presence of pulmonary cavitation, the therapeutic effect of isoniazid is enhanced about equally by daily streptomycin or PAS. Higher isoniazid dosages may produce even better results, but the evidence for this is still quite inadequate. Except in quite unusual situations, corticotropin and corticosteroids are safe additions, but have not been shown to add appreciably to the end results. Pyrazinamide and cycloserine are of value in certain failures with other drugs, despite their appreciable hazards. Thiocarbanidin and kanamycin have as yet shown little value in patients, despite the encouraging results in animal experiments (Author's summary).

M. J. Small

PULMONARY PHYSIOLOGY

The Average Density of Healthy Lung. J. Fowler* and A. Young. *Am. J. Roentgenol.*, February, 1959, 81: 312–315. (*King's College Hosp., London, England)

A survey is given of values for lung density quoted by various writers together with a method of measuring the density by integrating the proportion of air space in a histologic section. This method gives 0.26 ± 0.03 gm. per cm. for the average density of healthy lungs, in agreement with anatomic evidence (after authors' summary).

T. H. Noehren

A Study of the Mechanism by Which Exercise Increases the Pulmonary Diffusing Capacity for Carbon Monoxide. J. C. Ross,* R. Frayser, and J. B. Hickam. *J. Clin. Invest.*, June, 1959, 38: 916–932. (*Indiana U. School of Med., Indianapolis)

A study has been made of the means by which exercise increases the pulmonary diffusing capacity for carbon monoxide (D_L) in normal subjects, using both the steady-state and breath-holding techniques. Increases in cardiac output produced by means other than exercise caused no significant change in D_L as measured by either the steady-state or breath-holding techniques. Steady-state D_L was found to be very sensitive to the ventilation rate and, by hyperventilation alone, D_L was increased as much as it was by exercise at the same minute ventilation. Breath-holding D_L was not affected by hyperventilation preceding the measurement. Although the effect of increased ventilation rate could explain the increase in steady-state D_L with exercise, an adequate explanation for the increase in breath-holding D_L which occurs during exercise was not obtained.

E. Dunner

Physiological Effects of Chronic Exposure to Carbon Monoxide. S. S. Wilks,* J. F. Tomashefski, and R. T. Clark, Jr. *J. Appl. Physiol.*, May, 1959, 14: 305–310. (*School of Aviation Med., USAF, Randolph A. F. Base, Tex.)

A group of 7 dogs were given a daily 6–8-hour exposure to CO levels of 0.08 to 0.10 per cent for a period of 36 weeks, and their tolerance to CO was compared with that of normal dogs, altitude-acclimatized dogs, and dogs transfused with blood from normal dogs. The observed increased tolerance to CO was attributed primarily to the increase in concentration of the hemoglobin pigments of the blood. With this increased hemoglobin, there was a greater reserve of the pigment for O_2 transport at a given concentration level of HbCO. The time required to reach an equilibrium level with a given P_{CO} was greater for the acclimatized than for the normal animals. The Pa_{O2} was always lower than PA_{O2} during the acclimatization period. There was no evidence of factors, other than hematologic, which increased the tolerance of the dogs to CO. Animals subjected to other hypoxic stress such as altitude, or normal animals transfused with normal blood to increase the hematocrit level, showed increased tolerance similar to that accomplished by chronic exposure to low CO levels (Authors' summary).

A. L. L. Bell, Jr.

Oxygen Cost of Voluntary Hyperventilation. J. F. Murray.* *J. Appl. Physiol.*, March, 1959, 14: 187–190. (*U. of California, Los Angeles)

The oxygen cost of voluntary hyperventilation was measured using an open-circuit technique with three variations: unaided hyperventilation of air, breathing through an increased dead space, and adding carbon dioxide to the inspired air. After a given minute volume had been maintained for 10 minutes, the oxygen consumption was the same with the three methods in spite of marked differences in the respiratory exchange ratio and volume of carbon dioxide produced. The mean oxygen cost for all three methods was 3.2 ml. per liter of ventilation. The amount of non-metabolic oxygen stored during the first minute of hyperventilation was estimated by finding the difference between the oxygen uptake during the first minute and the amount utilized when a steady state is reached. It is concluded that the effects of changing oxygen stores are minimal after 10 minutes of hyperventilation, and probably after

5 minutes, at a constant minute volume (after author's summary).

A. L. L. Bell, Jr.

The Primary Hypoventilation Syndrome. T. Rodman* and H. P. Close. *Am. J. Med.*, May, 1959, 26: 808–817. (*Philadelphia V. A. Hosp., Penn.)

In this paper, a fifth patient with the "primary hypoventilation syndrome" without obesity is reported. The findings in the other 4 patients and in alveolar hypoventilation in general are reviewed. Special studies were carried out to clarify the defect in the respiratory regulatory mechanism. These studies demonstrated a totally absent response to hypoxia and lobeline, and a slightly diminished response to CO_2 with improved oxygenation. The findings are compared to those in experimental animals in which the afferent nerves from the chemoreceptors are severed.

The most prominent clinical feature of patients with this syndrome is polycythemia. The differentiation between primary and secondary polycythemia is often relatively clear from the clinical picture. There may be an obvious cause of arterial oxygen unsaturation, or the presence of leukocytosis, thrombocytosis, and splenomegaly may suggest that the hematologic problem is a primary one. It seems likely, however, that unless oxygen saturation studies are carried out, cases of the primary hypoventilation syndrome, in which the presenting picture is that of polycythemia, will be misdiagnosed as primary polycythemia.

T. H. Noehren

MICROBIOLOGY AND IMMUNOLOGY

Metabolic Characteristics of Yellow Bacilli (the So-Called Atypical Acid-fast Bacilli) and Their Significance in the Differentiation of Various Acid-fast Bacilli (in Japanese). K. Oshima.* *Kekkaku*, June, 1959, 34: 400–405. (*Toneyama National Hosp., Osaka, Japan)

In view of the absence of definite bacteriologic or pathologic differentiation of the so-called "yellow" bacilli from other acid-fast bacilli in some cases, attempts were made to classify them by means of their metabolic characteristics.

(*1*) The metabolic activities of yellow bacilli (scotochromogenic) were generally low, and no increase of oxygen uptake was observed by the addition of benzoic acid derivatives which were easily metabolized by other acid-fast bacilli. Oxygen uptake by yellow bacilli was most remarkable in the presence of glycerol among the sugars and organic acids. But far more increased oxygen uptake was seen when higher hydrocarbons, especially *n*-dodecane, were added. (*2*) *Mycobacterium avium* Takeo and *M. 607* strains consumed large amounts of oxygen when benzoic or salicylic acid was added, but oxygen uptake in the presence of hydroxybenzoic acid or *n*-dodecane was negligible. The metabolic behaviors of the BCG and H37Ra strains resembled those of the avian strains although the activities were low. Oxygen uptake of *M. phlei* was always high in the presence of benzoic, salicylic, or *p*-hydroxybenzoic acid, or *n*-dodecane.

In consequence, the author considers the measurement of metabolic activities helpful in classifying atypical and other acid-fast bacilli when these four substances and glycerol are used as substrates for respiration.

I. Tateno

The Effect of Surface Moisture on Colonial Morphology of BCG. P. Farmer.* *Tubercle*, April, 1959, 40: 121–123. (*Glaxo Lab. Ltd., Greenford, Middlesex, England)

Mycobacterium tuberculosis on solid media shows variations in colonial morphology; "rough" and "smooth" colony types occur. The ability of virulent strains to grow in serpentine colonies was first illustrated by Koch in 1884. More recently, Middlebrook and others related the microscopic arrangement of cells in these serpentine cords to virulence. In virulent strains the cells were found to be orientated in parallel along the cords, whereas in the avirulent strains a less orientated arrangement occurred.

Experiments in this laboratory with various substrains of BCG have shown that the appearance of serpentine colonies on blood agar medium can be influenced by the physical environment. Cultures normally growing as discrete colonies will form serpentine colonies on media with excess surface moisture. Under microscopic examination it was seen that the cords formed by the "rough" variants consisted of cells more tightly held together than the "smooth" variants. This correlated with results from experiments in guinea pigs (Jensen tests) in which the former caused larger local lesions than the latter. The Moreau substrain was found to form only loose cords. This observation correlates with the reported lower invasiveness of this substrain in man.

M. J. Small

EXPERIMENTAL PATHOLOGY

Experimental Cerebral Embolism with Antifoam A. J. K. Penry, A. R. Cordell, F. R. Johnston, and M. G. Netsky. *J. Thoracic Surg.*, March, 1959, 37: 342–351.

One of the greatest drawbacks to the bubble oxygenator has been the difficulty in defoaming blood adequately. The passing of blood over surfaces coated with a methyl polysiloxane resin

(Antifoam A) is the most commonly used method. It is highly effective as a defoaming agent in concentration of 1 to 10 parts per million if sufficiently dispersed. The term "concentration" implies that a small amount of the material must be present in the diluting medium. There is, therefore, no support to the concept that adherence alone to a debubbling surface is adequate for defoaming. A small amount of antifoam is necessarily present in the defoamed blood. Many investigators have suspected that antifoam causes some of the cerebral complications observed with the use of this material in bubble oxygenators. Experiments were therefore undertaken to observe the cerebral effects of Antifoam A when injected into the internal carotid artery of the dog. Abnormal clinical signs and histologic changes ranging from neuronal changes to infarcts with hemorrhage were found in all animals receiving an antifoam. Capillary occlusion by antifoam can be demonstrated by the oil red 0 technique in frozen sections, and differentiated from fat by the osmic acid method. The clinical and pathologic findings in cerebral embolism with antifoam are comparable in many respects to those observed in cerebral fat embolism. The cerebral distribution of the emboli is interesting, and it appears that the angle of branching and the size of the main division influence the distribution of these emboli.

R. E. MacQuigg

Spontaneous Diseases and Their Control in Laboratory Animals. R. T. Habermann. *Pub. Health Rep.*, February, 1959, 74: 165–169.

Losses of laboratory animals due to diseases can be reduced considerably by good animal husbandry practices and sanitation, and by the development of effective methods of treatment and control. Sulfamerizine given daily in the feed eliminated endemic pneumonia in the third, fourth, and fifth generations of rats. Giving the drug at a daily dose rate of 5 mg. in 20 gm. of ground rat feed may be a good, practical way to obtain a rat colony free of chronic respiratory disease.

E. Dunner

The Effect of Vitamin A on Tracheal Epithelial Regeneration and Its Significance in Influenzal Pneumonia (in German). H. Otto and J. Kiwi. *Klin. Wchnschr.*, February, 1959, 37: 203–207.

In influenza epidemics, involvement of the respiratory organs by the infection is pronounced. In autopsy examinations of persons who have succumbed to the disease, it has been noted that the respiratory epithelium is destroyed. Its subsequent metaplastic regeneration—that is, replacement by pavement epithelium—was usually followed by an acute bronchopneumonia. This regenerated epithelium is without cilia, a circumstance that cannot be overlooked in the development of an infection in the bronchial tree.

Ninety-six rats were exposed to air mixed with formaldehyde gas which caused destruction of the ciliated epithelium in the bronchial tree. The rats which were subjected to high doses of vitamin A intake were noted to regenerate ciliated epithelium, while the control animals were found to have metaplastic epithelium. This metaplastic regeneration was completely missing in the vitamin A–treated animals.

The experiment suggests that vitamin A treatment may be useful in influenzal tracheobronchitis.

Z. Virágh

Effect of Dietary Proteins and Amino Acids on the Susceptibility of Mice to Bacterial Infections. R. J. Dubos* and R. W. Schaedler. *J. Exper. Med.*, July, 1958, 108: 69–81. (*Rockefeller Institute, N. Y., N. Y.)

To illustrate the influence of nutritional intake of proteins and amino acids upon susceptibility to bacterial infections, groups of young albino mice were fed four different types of diets and compared as to rate of weight gain and resistance to experimental infections with *Staphylococcus aureus*, *Mycobacterium fortuitum*, or *M. tuberculosis* var. *bovis*. Detailed composition and analyses of the diets are presented; in general, they contained: 21 per cent "crude" protein, 20 per cent casein, 8 per cent casein, or 8 per cent casein supplemented with 12 per cent of a mixture of essential amino acids. There was no relation between the effects of the diets on weight curves of the animals and on resistance to infection. The differences in susceptibility of animals on the four dietary regimens were the same regardless of bacterial pathogen, size of infective dose, or duration of disease. Mice on the diet containing 8 per cent casein were the most susceptible; those on the same diet supplemented with amino acids, the most resistant. The results suggest that qualitative characteristics of dietary protein are as important as the quantity of protein intake in determining susceptibility to infection.

C. H. Pierce

The Erythropoietic Factor in Hypoxic Patients with Emphysema without Secondary Polycythemia. W. H. Whitcomb, R. M. Bird,* P. C. Johnson, J. F. Hammarsten, and M. Moore. *A.M.A. Arch. Int. Med.*, June, 1959, 103: 871–875. (*U. of Oklahoma School of Med., Oklahoma City)

Experiments employing a conventional rat assay method for estimating plasma erythro-

poietin activity indicate that the plasma from patients who are hypoxic as a result of pulmonary emphysema, but who have not developed secondary polycythemia, contain quantities of erythropoietin greater than those seen in normal subjects. There is evidence that the failure of emphysematous and hypoxic patients to develop the expected polycythemia results from a refractory bone marrow.

W. J. STEININGER

Studies on Tuberculin Fever. I. The Mechanism of Fever in Tuberculin Hypersensitivity. C. H. HALL, JR.,* and E. ATKINS. *J. Exper. Med.*, April, 1959, 109: 339-359. (*U. of Minnesota Med. School, Minneapolis)

This investigation was undertaken to determine whether the febrile response to Old Tuberculin (OT) in sensitized animals is a specific property of the hypersensitivity system or is due to trace amounts of endotoxins contaminating the OT antigen.

Rectal temperatures of rabbits sensitized to BCG were compared after the animals had been tested in various ways with OT and typhoid endotoxin. The following results eliminated the presence of a contaminating endotoxin as a contributory factor toward the fever-enhancing property of OT: (*1*) Doses of OT much larger than those causing high fever in hypersensitive recipients failed to produce a febrile response in normal animals. (*2*) *In vitro* incubation of OT with serum did not accelerate fever in hypersensitive recipients nor augment the minimal febrile response of desensitized animals. (*3*) Thorotrast failed to potentiate the response of normal animals to OT or to reverse the "tolerance" of desensitized recipients. (*4*) Hypersensitive animals rendered pyrogen-tolerant showed no significant diminution in their response to OT. Conversely, repeated daily injections of OT did not confer tolerance to a small dose of typhoid vaccine. Passive serum transfer demonstrated the presence of a circulating pyrogen during tuberculin fever which was indistinguishable from endogenous pyrogens obtained in other types of experimental fever and was clearly differentiated from OT itself.

The authors postulate, on the basis of these findings, that tuberculin fever is due to a circulating endogenous pyrogen released by a specific action of OT on sensitized cells of the host.

C. H. PIERCE

Inhibition of Tuberculin Skin Hypersensitivity in Guinea Pigs by Injection of Tuberculin and Intact Tubercle Bacilli During Fetal Life. D. W. WEISS.* *J. Exper. Med.*, July, 1958, 108: 83-104. (*U. of California, Berkeley)

Female guinea pigs were subjected to laparotomy at different stages of pregnancy and their fetuses were injected through the uterine walls with one of the following preparations: Old Tuberculin (OT), tubercle bacilli of the BCG strain killed by heat or exposure to phenol, and living BCG. A large number of the animals injected *in utero* with OT failed to develop skin hypersensitivity to PPD following vaccination with 200 or 800γ of phenol-killed tubercle bacilli (BCG) in early adulthood. Normal control animals were sensitized by vaccination with such quantities of phenolized BCG. The failure of animals which had been injected with OT in fetal life to respond hypersensitively to PPD after adult vaccination with tubercle bacilli is ascribed to their acquisition of a state of immunologic tolerance to tuberculoprotein (tuberculin tolerance).

Fetal injection with killed BCG conferred a state of tolerance on a few of the animals, and rendered others tuberculin sensitive. Fetal injection with living BCG sensitized most of the animals to tuberculin even when fetal exposure was as early as 46 days before birth, and it induced tolerance in none. Fetuses of the same litter, injected simultaneously with identical inocula, often responded differently, some becoming tolerant to tuberculin, others developing hypersensitivity, and still others remaining immunologically unaffected, becoming neither sensitive nor tolerant. The state of tuberculin tolerance induced in these experiments was limited. When tolerant animals were revaccinated with living BCG several weeks after vaccination with phenol-killed bacilli, they developed as high a degree of tuberculin skin sensitivity as the originally non-tolerant animals (after authors' summary).

C. H. PIERCE

Experimental Tuberculopneumoconiosis. P. GROSS,* M. L. WESTRICK, and J. M. MCNERNEY. *A.M.A. Arch. Indust. H.*, March, 1959, 19: 320-334. (*Mellon Institute, Pittsburgh, Penn.)

The inhalation of large quantities of so-called "inert" dusts in combination with large intratracheal infecting doses of avirulent tubercle bacilli produced tuberculopneumoconiotic lesions in guinea pigs. The dusts employed included a nonfibrogenic quartz (iron-contaminated), aluminum oxide, kaolin, and silicon carbide.

The ability of a dust to produce tuberculopneumoconiosis was measured by three criteria: (*1*) the extent and severity of the tuberculopneumoconiotic lesions produced; (*2*) in conjunction with a subminimal infecting dose of tubercle bacilli, the increase in the number of positive tuberculin reactors as compared with the number of such reactors in the nondusted infection controls; (*3*) in conjunction with a subminimal infecting dose of tubercle bacilli, the incidence of animals with tuberculopneumoconiotic lesions as compared with the incidence of animals with such lesions in a group exposed to a standard dust such as quartz (high standard) or kaolin (low standard). Judged by these criteria, the potential of iron-contaminated quartz to produce tuberculopneumoconiosis was considerably greater than that of the other dusts, and silicon carbide was least able to produce this lesion.

From a pathogenic study of the experimental lesion it is concluded that tuberculopneumoconiosis is basically a chronic obliterative alveolitis with fibrous replacement of normal tissues. Central necrosis, if present, is a secondary phenomenon. The question is raised whether there exists any dust—even of the so-called "inert" type—which in conjunction with a tuberculous infection may not produce tuberculopneumoconiosis, provided that the concentrations of dust in the lung are sufficiently large and the virulence of the infecting organism is adequate (Authors' summary).

T. H. Noehren

The Effect of Treatment with a Large Dose of Isoniazid on an Established Tuberculous Infection in Mice. J. M. Robson* and F. M. Sullivan. *Brit. J. Pharmacol.*, June, 1959, 14: 222–228. (*Guy's Hosp. Med. School, London, England)

Mice were infected intracorneally with *M. tuberculosis* var. *bovis* and the infection was allowed to develop for a period of 2 weeks. At this stage the animals were divided into 2 main groups: 1 received no treatment, the other was treated with large doses of isoniazid (3.0 mg. per mouse per day). The effect of treatment on the primary lesion, the viability of the bacilli, the systemic spread of infection, and the production of immunity were observed. Treatment was continued for 11 months, after which the animals were observed for another 8 months. Within a few days of starting isoniazid treatment, the primary lesion stopped increasing in size, regressed slightly, and stabilized at a size of about ⅓ of that of the controls. There was little evidence of any clearing of the bacilli from the lesions; they remained strongly acid-fast and morphologically normal for many months after infection, although by 13 months about half of the organisms in both groups had become very granular.

The evidence suggests that in control and treated animals the bacilli in the cornea had usually all died within 8 months after infection. In 2 treated corneas, living virulent bacilli were demonstrated 15 months after inoculation. In the untreated animals, the disease spread systemically to involve the lungs, liver, and spleen, and by 1 year after inoculation the systemic tuberculous infection was very heavy, though not enough to kill the animals. The lesions in these animals contained many acid-fast bacilli. In the treated group, systemic spread of infection as judged by the development of small macroscopic lung foci was slight; acid-fast bacilli were found in only 1 animal. Immunity was measured by the resistance of the other eye to reinfection. The controls all showed such immunity, but in the treated group no immunity could be detected after 1 year of treatment, and practically none at 5 months after the initial inoculation.

E. Rothstein

Comparison of the Granuloma Producing Capacity of Normals and Sarcoid Granuloma Patients: Experimental Analysis of the Sarcoid Diathesis Theory. H. J. Hurley* and W. B. Shelley. *Am. J. M. Sc.*, June, 1959, 237: 685–692. (*Hahnemann Med. College, Philadelphia, Penn.)

In an attempt to evaluate the significance of the theory of the sarcoid diathesis in the production of sarcoid granulomas, the granuloma-producing capacity of 300 normal male subjects was compared with that of 35 patients with sarcoidosis and 6 patients with zirconium deodorant granulomas. The granulomagenic stimuli employed were intradermal injections of sodium stearate, beryllium, silicon, zirconium, homologous whole blood, et cetera. There was no variation, qualitatively or quantitatively, between the 300 normal subjects and the others to any of the stimuli except for the water-soluble zirconium salt in the case of the patients with zirconium deodorant granulomas. At least in sarcoidosis and zirconium deodorant granuloma, it is doubtful that the sarcoid mode of reaction is operative. In these two conditions and probably in other varieties of sarcoid granulomas, a specific granulomagenic substance is responsible for the production of this unusual tissue response. Continued search for such substances is indicated.

W. J. Steininger

The Fate of Inhaled Particulates in the Early Postexposure Period. H. Brieger* and C. W. LaBelle. *A.M.A. Arch. Indust. H.*, May, 1959, 19: 510–515. (*Jefferson Med. College, Philadelphia, Penn.)

After exposure of rats for 7 hours to an atmosphere containing insoluble dye particles, redistribution of particles took place in 3 phases. Initially, the largest amount of dye was found on the skin. During a transitory second phase of 10 to 14 days, very large quantities passed through the gastrointestinal tract, including material transported from the lungs by secondary mechanisms. In the last phase, dye was found only in the lungs. The true site of exposure thus changed from the skin to the gastrointestinal tract to the lung. The importance of each of these areas of contact depends on the properties of the noxious agent involved.

W. J. Steininger

SYMPOSIA

***A.M.A. Arch. Indust. H.*, February, 1959, 19:**

Uses of Beryllium and Its Compounds. A. R. Kaufman.* Pp. 91–93. (*Nuclear Metals, Inc., Concord, Mass.)

At the present time there is considerable, and apparently increasing, interest in beryllium and its uses. This interest, which is directed not only to the metal itself but also to some of its compounds, is based on the fact that beryllium has certain mechanical properties that make it an especially interesting engineering material. Its nuclear properties make it useful in other applications.

It appears that enough is being learned about beryllium so that, if it is converted into an optimal metallurgic state and if the designers go more than half way to meet the material, it can perhaps be used, at least to a limited extent. The sorts of health problems that will arise are not concerned with the companies producing beryllium, because they have been well aware of the toxic hazard for years and presumably have it under control. There is, however, concern that when beryllium is put into structures of one sort or another or sent to fabrication plants, the men doing the work either of assembly or repair may be endangered. It appears, however, that adequate indoctrination and proper industrial hygiene could prevent such hazard.

Exposure Data and Epidemiology of the Beryllium Case Registry—1958. M. F. Peyton* and J. Worcester. Pp. 94–99. (*Harvard School of Public Health, Boston, Mass.)

The Beryllium Case Registry at the Massachusetts General Hospital contained 606 cases of the acute and/or chronic pulmonary form of beryllium poisoning as of June, 1958. Since the Registry's inception in 1952, cases have been collected from all parts of the United States and the data organized for interpretation and follow-up.

From studying the 606 cases in the Beryllium Case Registry and making a more complete study of 250 of these cases where some information was available as to the number exposed, the following conclusions can be drawn: (*1*) There is a marked reduction in cases with exposure after 1949. It would appear that the controls which were introduced in 1949 have been effective. (*2*) There has been a three- to fivefold increase in the number of cases reported by industry in the past 10 years. (*3*) The attack rate among the exposed population studied ran between 5.8 per cent and 7.5 per cent, and the case fatality rates ran between 5.8 per cent and 23 per cent for the 13–20-year period under study. (*4*) In one plant there was an increase in the attack rate for the acute cases with increasing length of employment. (*5*) There is some evidence that the morbidity rate for the chronic cases is higher among women than among men.

Intelligent planning and cooperation between industries and those undertaking an epidemiologic study in the future are essential to solving the problem of beryllium poisoning.

Case Data from the Beryllium Registry. T. C. Hall,* C. H. Wood, J. D. Stoeckle, and L. B. Tepper. Pp. 100–103. (*Mass. General Hosp., Boston)

Most of the reported cases of chronic beryllium disease have had an exposure of more than 2 years' duration. Nevertheless, there is evidence, based on careful study of the work record, that one worker acquired the chronic illness after a heavy exposure of less than 6 hours' duration.

Chronic beryllium disease has commonly been precipitated or exacerbated by some stress. The disease itself has shown a variety of symptomatic manifestations. The respiratory tract is usually primarily involved, with symptoms of cough and dyspnea predominating. Nine of the cases in the Registry have never developed symptoms of chronic disease, but are included on the basis of the roentgenographic evidence and positive beryllium assays. There is good evidence that chronic beryllium disease is a systemic illness and may involve other body organ systems. Cases of splenic and hepatic enlargement without other known cause are recorded, and autopsy evidence of liver, spleen, and lymph node involvement is available. There is frequent alteration in the serum protein distribution, and major weight loss is common. Striking is the fact that 31 chronic patients, or 10 per cent of those where data are adequate, have developed renal stone.

In addition to renal stone, spontaneous pneumothorax has been a complication, involving 37 of the chronic cases, or about 12 per cent of those under long-term observation. Not more than 4 patients have been reported to have active pulmonary tuberculosis. (Of the 104 tuberculin skin tests reported, 24 per cent have been positive.) Lung cancer has been recorded only once. Approximately 20 cases have been recorded in which a diagnosis of rheumatoid arthritis has been made at the onset, or in which migratory joint pains have appeared in the course of chronic beryllium disease.

Beryllium assays have been conducted in about $^2/_3$ of the chronic patients. Tissue determinations have been positive in 80 per cent of these cases, while blood determinations have been positive in only 13 per cent. An examination of lung tissue in controls—persons not known to have ever been exposed to beryllium—has revealed that minute but nevertheless spectrographically readable amounts of the element may occasionally be detected. This finding means that a definite diagnosis of chronic beryllium disease requires characteristic pathologic changes in addition to a positive beryllium assay.

It is instructive to examine the case records (all of them complete) submitted by a single contributing physician. Of 55 living patients, approximately $^1/_2$ are gainfully employed or engaged in full-time homemaking. Of these, about $^2/_3$ are asymptomatic and are receiving no treatment; the others are on a steroid program because of symptomatic chronic beryllium disease. Of these patients who are incapacitated, the majority are able to "putter" around the house, avoiding exertion. Two are entirely confined to bed despite a constant steroid program.

Long-Term Experience with Beryllium Disease. E. M. Kline* and Thomas W. Moir. Pp. 104–109. (*Western Reserve U., Cleveland, O.)

A report of 20 patients with chronic beryllium disease who have been followed for a minimum of 5 years is presented. A difference in attack rate, duration of exposure, and latent period between those exposed only to the zinc beryllium silicate compound in the lamp factories and those exposed to the constituents of the phosphor during its chemical processing indicates the importance of other pathogenetic factors, in addition to a sensitivity mechanism of the type proposed by others.

Long-Term Experience with Beryllium Disease. J. M. De Nardi.* Pp. 110–116. (*Western Reserve U., Cleveland, O.)

Studies based on long-term experience with beryllium disease are presented, and show the following: (*1*) apparent adequate control of occupational, acute, disabling disease occurrence since 1953; (*2*) incremented incidence data on the chronic form of disease since 1953; (*3*) the present clinical status of a major number of the personnel having had an acute form of occupational respiratory disease; (*4*) the concentration of incidence of the chronic disease in the basic industry by exposures in certain years; (*5*) an unusual hour-exposure factor in the basic industry experience; (*6*) an abrupt cessation of the incidence of chronic disease in the basic industry, both directly and indirectly, since completion of industrial hygiene controls; (*7*) a predilection for incidence of chronic disease in women; (*8*) a seemingly greater morbidity on incidence in men; and (*9*) the beneficial effect of steroid therapy on the chronic disease (Author's summary).

Radiological Abnormalities in Chronic Pulmonary Disease Due to Beryllium. J. E. Gary* and R. Schatzki. Pp. 117–125. (*1180 Beacon St., Brookline, Mass.)

The concept that there are stages of involvement of the lung by berylliosis which can be identified from films is questioned. Rather, it is believed that the lung responds in one or another way to this irritant. Types of disease rather than stages of disease seems a concept more likely to depict the true state of affairs. There are frequently marked contrasts in the severity of disease as judged from the roentgenographic appearance, the clinical condition of the patient, or by pulmonary function tests. The importance of serial films of persons known to have been exposed to beryllium is stressed if an early diagnosis is to be made. The question of treatment of persons who show roentgen abnormalities in the absence of symptoms is raised. Cases with film changes for over 5 years with no symptoms have been seen.

Differential diagnosis is mentioned only briefly since it is believed that this is impossible from films alone. A definitive diagnosis demands the finding of significant amounts of beryllium in tissue showing characteristic pathologic changes. No case has been seen which shows clinical evidence of berylliosis with a normal chest film.

Radiological Diagnosis of Chronic Beryllium Disease. G. W. Chamberlin.* Pp. 126–131. (*Reading Hosp., Penn.)

Twenty-five patients from a community near a beryllium extraction and fabrication plant showed clinical and roentgenographic evidence of chronic beryllium disease. All of these patients had common signs, symptoms, and laboratory and roentgen evidence. As a group, they conformed to those cases previously reported in the literature incident to the inhalation of beryllium. One conspicuous difference in this series was the relatively large number of patients with secondary or neigh-

borhood exposure. Of the 25 patients, only 4 were employees; 7 patients had contact with clothing of employees of a beryllium plant, and 3 of these also lived in the adjacent neighborhood; 4 patients lived within a radius of $1\frac{1}{2}$ miles, and 10 lived more than $1\frac{1}{2}$ miles and less than $6\frac{1}{2}$ miles distant.

Because of this unusual distribution of cases, it was of interest to investigate the possibility of incidental beryllium in the lungs of other people in the community. Spectroscopic examination revealed that beryllium was present in the lungs of 12 of 13 routine autopsy specimens. As far as could be determined, none of these patients had clinical evidence of berylliosis. While this evidence needs further confirmation in a larger series, it suggests that some beryllium is being inhaled by a large segment of the population. This would account for the presence of chronic granulomatous disease in those patients in the series who have no recognizable exposure but who do have reasonably characteristic clinical, roentgenographic, and histologic evidence of the disease.

Respiratory Pathophysiology in Chronic Beryllium Disease. E. A. Gaensler,* J. M. Verstraeten, W. B. Weil, D. W. Cugell, A. Marks, J. B. Cadigan, R. H. Jones, and M. F. Ellicott. Pp. 132–145. (*Tufts U., Boston, Mass.)

Respiratory pathophysiology was studied in 30 patients with chronic beryllium disease. Observations included conventional ventilatory function studies, lung volumes, gaseous distribution, physiologic dead space and alveolar ventilation, diffusing capacity for carbon monoxide by both the single-breath and steady-state methods and, in some, oxygen diffusing capacity, pulmonary shunt, and exercise studies.

One-half, within the limitations of the measurements described, had normal ventilatory function, mechanics of breathing, gaseous distribution, and lung volumes. The remainder had varying degrees of "restrictive" insufficiency, although evidence of minor airway obstruction was common. Only 2 had moderate chronic obstructive emphysema. Overt cor pulmonale and polycythemia were not seen. All patients had low arterial oxygen tensions and incomplete saturation of arterial blood at rest. Because of varying degrees of hyperventilation, this bore no relationship to clinical signs and symptoms or to diffusing capacity.

The A-a gradient was markedly elevated during room-air and low-oxygen breathing at rest and during exercise. Previous work, suggesting "alveolar-capillary block" as the basic physiopathology in this disease, was confirmed: all but 2 patients had diminished D_{O_2} and D_{CO} measured during steady-state conditions at rest and during exercise. However, abnormal ventilation-perfusion relationship was shown to contribute significantly to the elevated A-a gradient; nearly all patients had an increased physiologic dead space, and the per cent shunt, whenever calculated, was increased.

Eleven patients were studied before and at varying intervals from 1 to $4\frac{1}{2}$ years during continuous steroid therapy. The typical response was one of moderate improvement of ventilatory function during the first year, with return to pretreatment values thereafter. There were no significant long-term changes of A-a gradient or diffusing capacity with treatment. At no time was treatment effective enough to cause return to normal of oxygen saturation, and the A-a gradient fell below 20 mm. Hg. However, no patient showed serious deterioration during the period of treatment, although 2 developed minor degrees of obstructive emphysema.

Physiologic data were compared with identical studies performed in a group of 45 patients with sarcoidosis. Although variations from the mean were considerable in both groups and, although the group of patients with sarcoidosis on the whole appeared to be less disabled, the average results of both groups were identical in every respect.

Pulmonary Function in Patients with Beryllium Intoxication. B. G. Ferris.* Pp. 146–149. (*Harvard U., Boston, Mass.)

Objective data should be collected to assess the effect of steroid treatment on pulmonary disease due to beryllium. The method of choice appears to be the alveolar-arterial oxygen pressure difference. Long-term studies on 4 patients with chronic beryllium intoxication indicate that either the steroid treatment has been inadequate or, despite "adequate" treatment, the disease has continued to progress; further studies will be required to resolve which of these is the correct explanation.

The Diagnosis of Beryllium Disease, with Special Reference to the Patch Test. G. H. Curtis.* Pp. 150–153. (*Cleveland Clinic, O.)

A survey of 37 cases of the respiratory syndromes of berylliosis, including 32 cases of chronic berylliosis, 8 cases of chronic fibrosing interstitial pneumonitis, 7 cases of sarcoidosis, and 4 cases of miscellaneous pulmonary diseases, was made with special reference to an evaluation of the patch test with soluble beryllium compounds as a diagnostic procedure. The patch test was compared with: (*1*) history of exposure, (*2*) pulmonary symptoms, (*3*) pulmonary function tests, (*4*) chest roentgenograms, (*5*) biopsy of the lung, (*6*) quantitative measurement of beryllium in the pulmonary tissue, and (*7*) the Kveim test.

The patch test could not stand alone as an absolute pathognomonic sign for the presence of

beryllium granulomatosis in the lungs, although a positive test was obtained in all 32 cases of chronic beryllosis. In 4 cases of pulmonary disease other than beryllosis but with a history of exposure, the diagnosis was based on procedures other than the patch tests, which did not permit an accurate interpretation of the pulmonary lesions.

In differential diagnosis of pulmonary diseases having similar clinical manifestations, a negative patch test and a history of nonexposure are dependable criteria for ruling out the presence of berylliosis.

The Diagnosis of Beryllium Disease, with Special Reference to the Patch Test. B. H. Waksman.* Pp. 154–156. (*Harvard U., Boston, Mass.)

Observations strengthen the suggestion that the lesions of beryllium disease may be hypersensitive reactions and that they deserve further study from this point of view.

The Differentiation of Sarcoidosis and Beryllium Disease. H. L. Israel* and M. Sones. Pp. 160–163. (*U. of Pennsylvania, Philadelphia)

Clinical and roentgenographic findings in 209 patients with sarcoidosis are compared with those encountered in beryllium disease. Roentgenographic examination of the chest, hands, and feet showed, in 68 per cent of the patients with sarcoidosis, changes which are not characteristic of beryllium disease. Clinical examination revealed an additional 12 per cent of extrapulmonary lesions which are not encountered, as a rule, in beryllium disease. Observation demonstrated complete roentgenographic clearing of pulmonary lesions in half of the remaining cases. Sarcoidosis and beryllium disease can be distinguished in most instances by roentgenographic and clinical criteria. Differentiation of the two diseases must rest on epidemiologic data, spectrographic analyses, and skin tests in only 10 per cent of cases (after authors' summary).

Treatment of Chronic Beryllium Poisoning. A. O. Seeler.* Pp. 164–168. (*Harvard U. Med. School, Cambridge, Mass.)

The data supplied to the Beryllium Case Registry concerning the effect of steroid therapy on chronic beryllium disease are reviewed. Although analysis of the data suggests that steroid therapy has a beneficial effect on the course of the disease, definitive evaluation of the role of steroid therapy must await further experience in the treatment of the disease.

Pathologic Changes of Beryllium Disease. J. B. Hazard.* Pp. 179–183. (*Cleveland Clinic, O.)

Exposure to beryllium-containing fumes of proper concentration produces an acute chemical pneumonitis. The pathologic findings in 6 patients with this disorder are reviewed. The various pathologic changes showed no correlation with the apparent duration of the disease. Thus, the patient dying with a severe proteinaceous edema of the lung without fibroplasia had a similar duration of illness (20 days) as did one of the patients with a severe organizing pneumonitis.

Though the changes were striking because of their severity, the pulmonary lesion had no specific character. A marked edema with heavy protein content of the edema fluid was like that seen in other forms of acute chemical pneumonitis. The intra-alveolar fibroplasia simulated that encountered in other forms of organizing pneumonitis. The fibrinoid membrane coincided in appearance with that found in viral pneumonitis or in uremic pneumonitis. The incidence of acute interstitial myocarditis was high, but the limited number of cases forbids interpretation of significance. The necrosis in the liver was of nonspecific character.

The Pathologic Changes of Chronic Beryllium Disease. H. R. Dudley.* Pp. 184–187. (*Harvard U. Med. School, Boston, Mass.)

Chronic beryllium disease may occur in many tissues, including the lungs, lymph nodes, liver, spleen, kidney, myocardium, skin, skeletal muscle, and pleura. The chronic reaction includes not only granulomas with or without birefringent crystals, conchoid bodies, and asteroids, but also a variable infiltration of lymphocytes and plasma cells. The latter may be responsible for many of the symptoms. The tissue response may represent a delayed sensitivity reaction and, as such, is not specific for beryllium disease. Frequent pulmonary emboli occur and may be related to debilitation, inactivity, and polycythemia (Author's summary).

Pathologic Changes Induced by Beryllium Compounds. A. J. Vorwald* and A. L. Reeves. Pp. 190–199. (*Wayne State U., Detroit, Mich.)

The changes induced by beryllium compounds are considered from two points of view, namely, the pulmonary tissue response provoked by beryllium oxide and beryllium sulfate in albino rats, and the mechanism possibly involved in that response. With respect to the tissue response, the compounds, either on intratracheal injection or on inhalation, have the capacity to induce chronic beryllium pneumonitis which resembles, in many respects, the pulmonary disease in human subjects. In addition, those compounds induce epithelial proliferation and primary pulmonary cancer in the exposed rats.

In regard to the mechanism, the studies reveal the following: (*a*) The soluble beryllium compound, beryllium sulfate, in human and "artificial" sera forms an insoluble precipitate, sug-

gestive of beryllium hydroxide and phosphate. (*b*) The effects of beryllium compounds on enzyme systems are quite specific, and several of these may have important implications in metabolic processes. (*c*) Within the cell, beryllium oxide has an appreciable effect on the cytoplasmic proteins and alters the RNA distribution, but has no demonstrable influence on DNA and the lipids (Authors' summary).

Medical Control of Beryllium. H. L. HARDY.* Pp. 203–204. (*Massachusetts Institute of Technology, Cambridge)

Experience suggests that the medical means of preventing beryllium disease in its chronic form are few. As in most occupational disease problems, prevention rests with ventilation engineers and the chemists who assay the beryllium-contaminated atmosphere. On the basis of current knowledge, there are a few useful rules for the physician. Because presently acceptable maximal allowable concentrations are low, and because it is always possible that there may be sudden overexposure, especially in new or previously untried operations, persons with demonstrable chronic respiratory disease should be excluded from all except background levels of beryllium. Workers with miliary chest film abnormalities of any cause, with or without symptoms, ideally are not allowed beryllium exposure, since changes in the roentgen picture after toxic beryllium exposure become difficult or impossible to interpret. Workers in whom beryllium poisoning has affected the lower respiratory tract should not be further exposed to toxic beryllium compounds.

The Analysis of Traces of Beryllium. J. CHOLAK.* Pp. 205–210. (*U. of Cincinnati, O.)

It is accepted generally that any method which is to be useful for the determination of beryllium in biologic materials must be capable of detecting, with accuracy, quantities of 0.10γ or less. The finding of such traces in tissues and body fluids is used frequently to support the diagnosis of beryllium disease. On the other hand, from time to time beryllium has been found in the lungs of persons who have had no history of occupational or "neighborhood" exposure to beryllium, and no beryllium disease. This paper discusses the reliability of the existing methods for determining trace quantities of beryllium and presents some data which deal with its occurrence in nature.

T. H. NOEHREN

DIFCO

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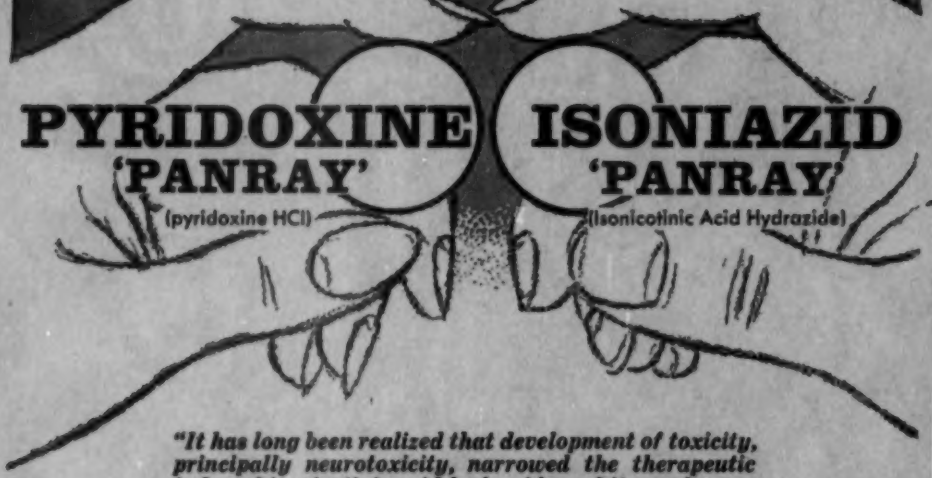

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[1]Bee View Bulletin 16:62 July '56 Tchertkoff, et al. JAMA 156:1549 (Dec. 25) 1954 Bicht & Villiger Am. Rev. Tbc 70:266 Aug. '54 Hughes, et al. N. Eng. J. of Med. 255 (2 11)-122, 1956 Carlson, et al.